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Title: The D614G mutation in Spike: increased infectivity and neutralizing Ab sensitivity and the underlying mechanism

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The D614G mutation in Spike: increased infectivity and neutralizing Ab sensitivity and the underlying mechanism

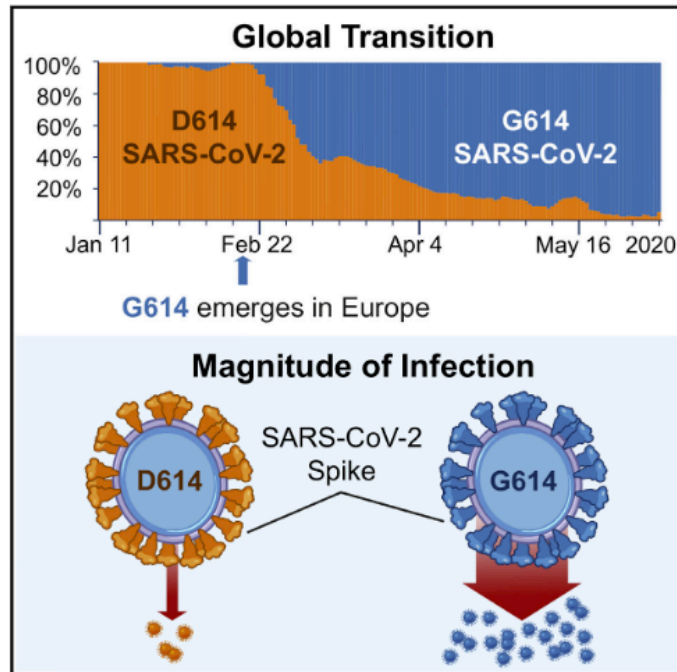


Los Alamos National Laboratory
August 26, 2020

Bette Korber
Los Alamos National Laboratory

Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus

Graphical Abstract



Highlights

- A SARS-CoV-2 variant with Spike G614 has replaced D614 as the dominant pandemic form
- The consistent increase of G614 at regional levels may indicate a fitness advantage
- G614 is associated with lower RT PCR Cts, suggestive of higher viral loads in patients
- The G614 variant grows to higher titers as pseudotyped virions

Authors

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In Brief

Korber et al. present evidence that there are now more SARS-CoV-2 viruses circulating in the human population globally that have the G614 form of the Spike protein versus the D614 form that was originally identified from the first human cases in Wuhan, China. Follow-up studies show that patients infected with G614 shed more viral nucleic acid compared with those with D614, and G614-bearing viruses show significantly higher infectious titers *in vitro* than their D614 counterparts.

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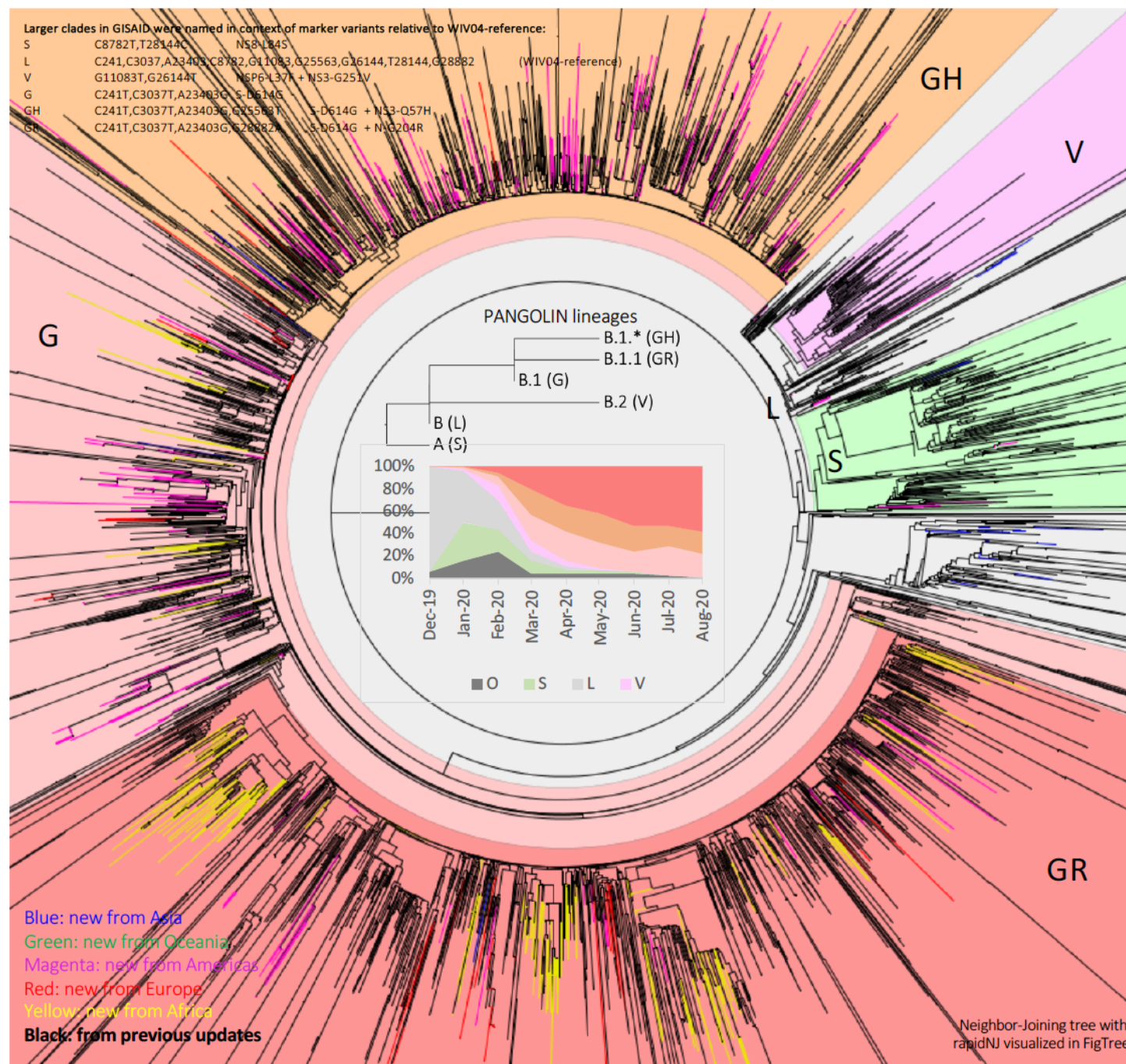


G614 is part of the G clade, which came out of Europe and has been followed by GISAID since early spring

The O, S, L, and V clade are rarely sampled after June 1,

G has two sublineages, GR and GH.

We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from GISAID on which this research is based. The original data are available from <https://www.gisaid.org>



Full genome tree derived from all outbreak sequences
2020-08-25

Notable changes:
77,909 full genomes (+971) (excluding low coverage, out of 84,426 entries)

Updated clades:
S clade 5,121 (+14)
L clade 3,869 (+7)
V clade 4,643 (+0)
G clade 18,101 (+227)
GR clade 24,764 (+372)
GH clade 17,850 (+324)
Other clades 3,561 (+27)

We gratefully acknowledge the Authors from Originating and Submitting laboratories of sequence data on which the analysis is based.



Overview: Cell paper

A single mutation in Spike
D614G has become the globally
dominant form of the virus

The G614 form is more infectious in
pseudotype virus assays

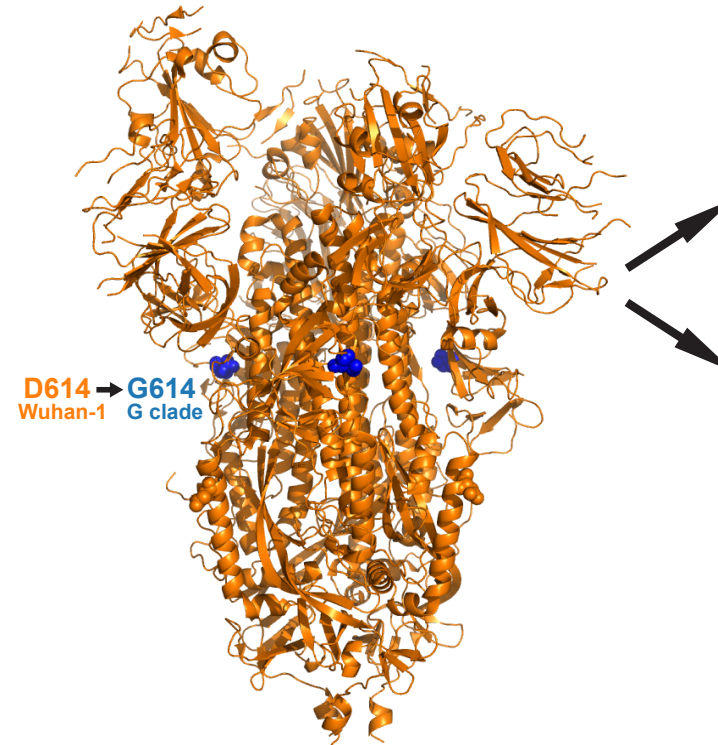
The G614 form is associated with higher
viral loads

G614 is not associated with increased
hospitalization

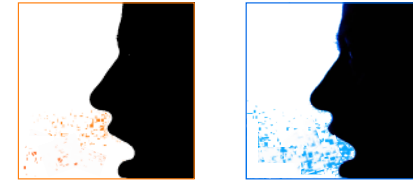
*New: G614 viruses are even more
sensitive to vaccine-elicited neutralizing
antibodies, convalescent sera, and NAb*
Weissman/Montefiori

*New: G614 has a preference for a “one-
up” RBD accessible conformation*
Gnanakaran/Acharya

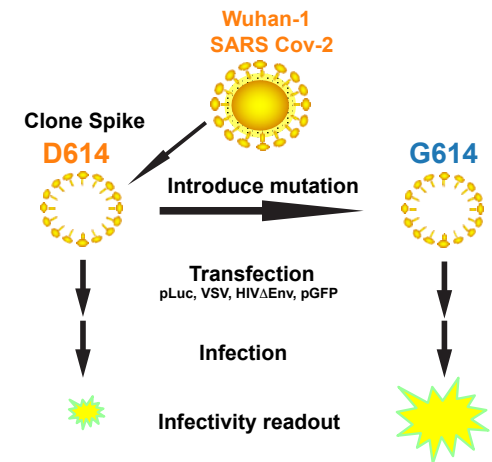
SARS-CoV-2 Spike



G614 is associated with higher viral loads in patients
indicated by lower RT PCR cycle thresholds for detection

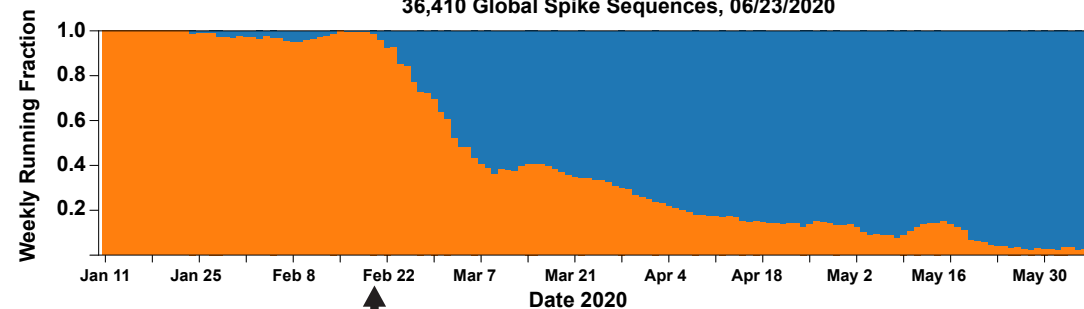


G614 is associated with higher infectious titers of
spike pseudotyped virus

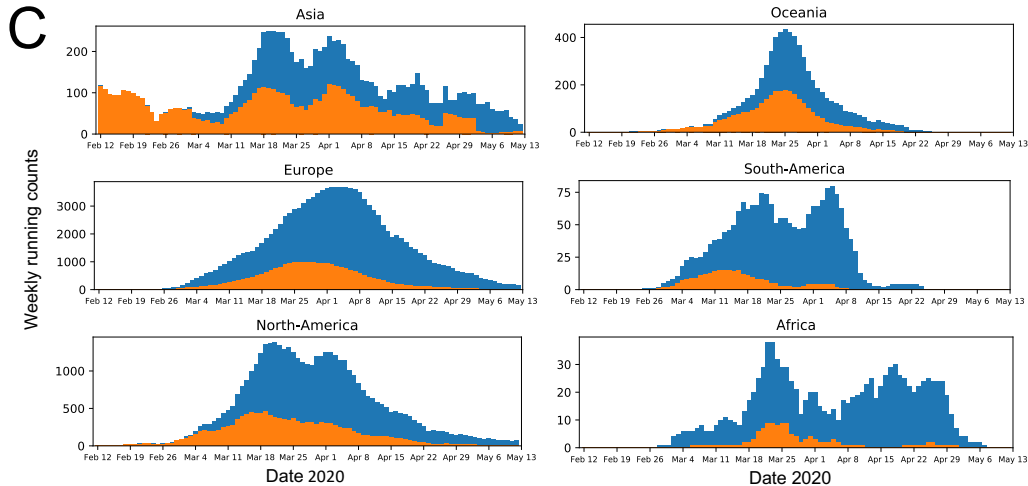
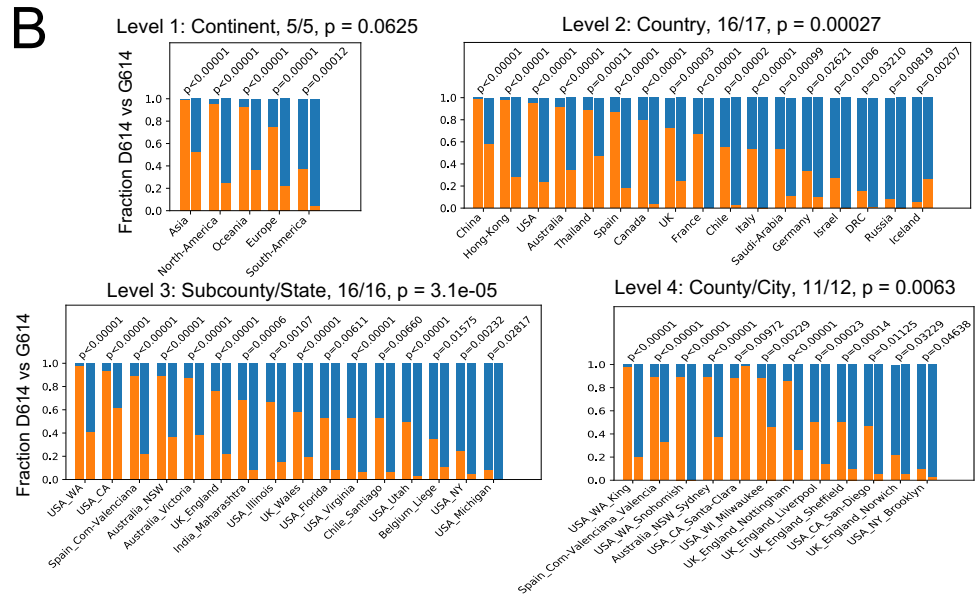
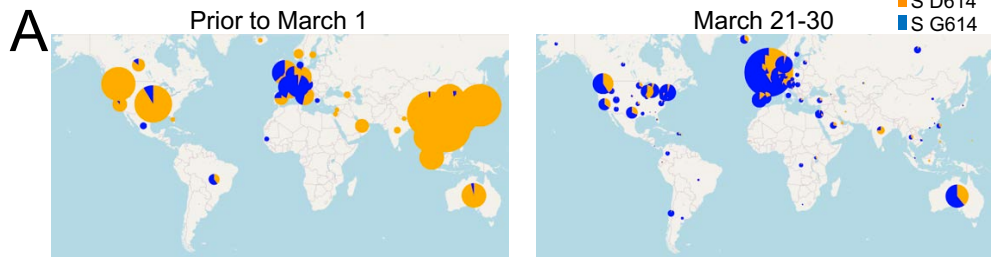


Global Transition from D614 to G614 variants

36,410 Global Spike Sequences, 06/23/2020



G614 emerges in Europe



Epidemiological evidence that the G614 clade is more transmissible

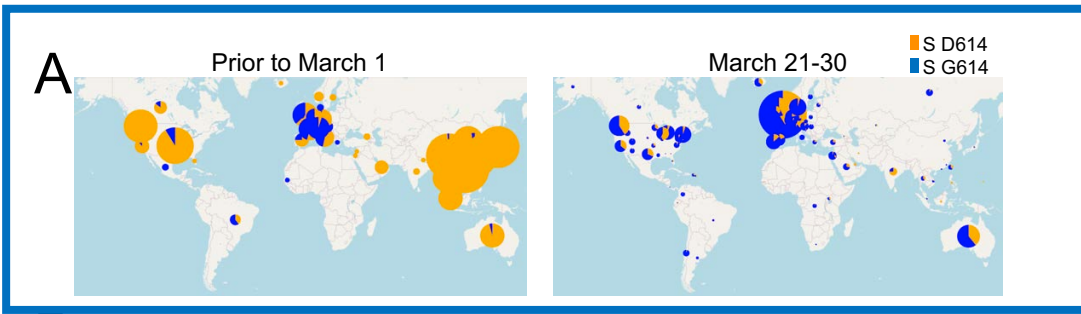
- 1) It very rapidly became the globally prevalent form
- 2) It increases in frequency *whenever* it enters a geographic region: Continent, country, sub-country, state, county, city
- 3) Recurring increases in frequency are *not* consistent with founder effects:

- If super-spreader events were common, they should be random, *and not always favor G614*

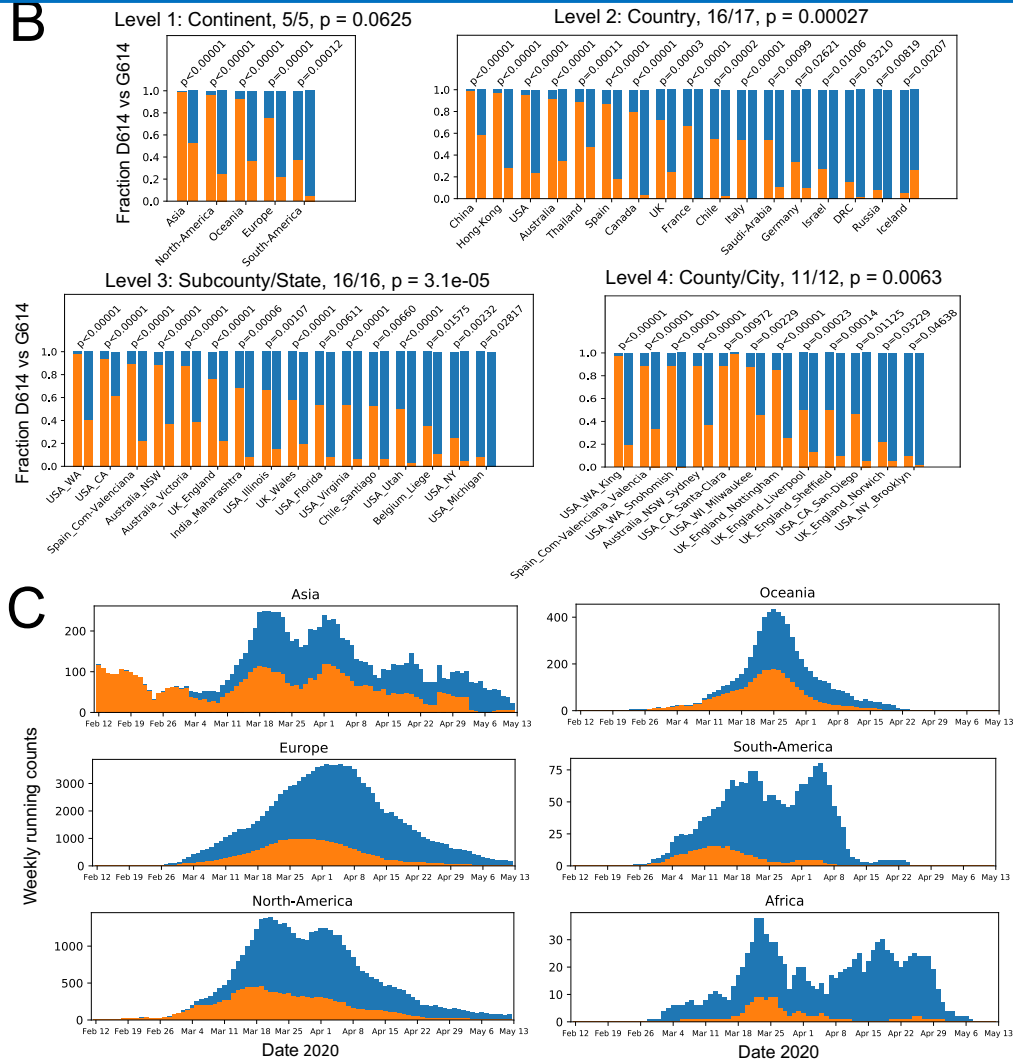
- Sampling biases should also be random *and not always favor G614*

- Multiple introductions from travelers *cannot* explain the advance of G614 in settings where it was first introduced into very well established epidemics

- Multiple introductions/super-spreader events cannot explain the repeated patterns of steady increase in G614 frequency well after stay-at-home orders are in place



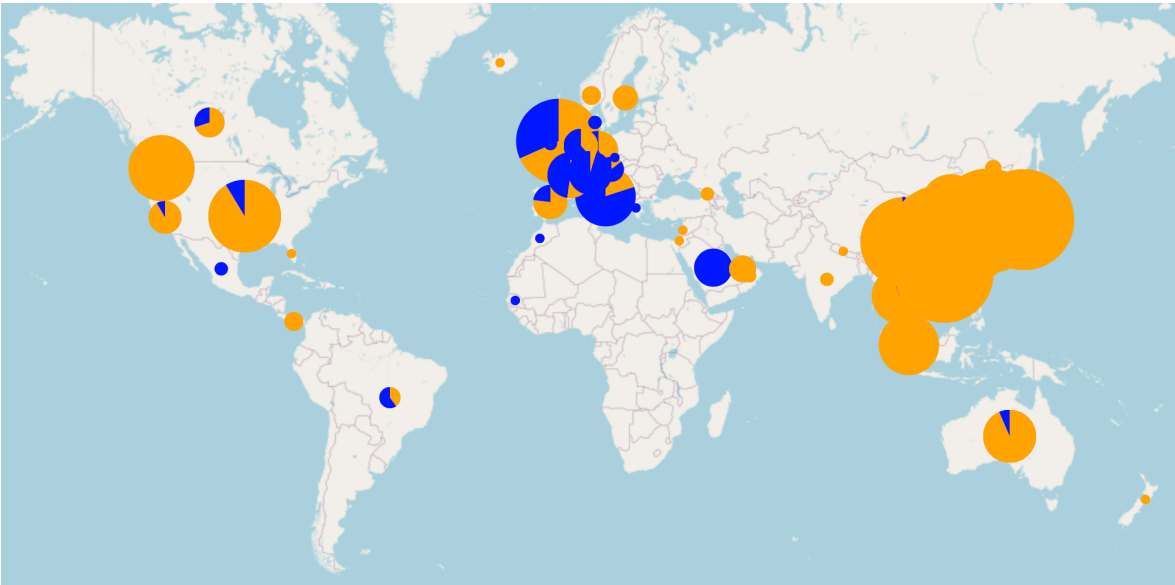
Maps of the world, with the original D614 form in orange, the G614 form in blue. The size of the circle indicates relative sampling frequencies, The pie slice indicates the frequency of the D and G forms.



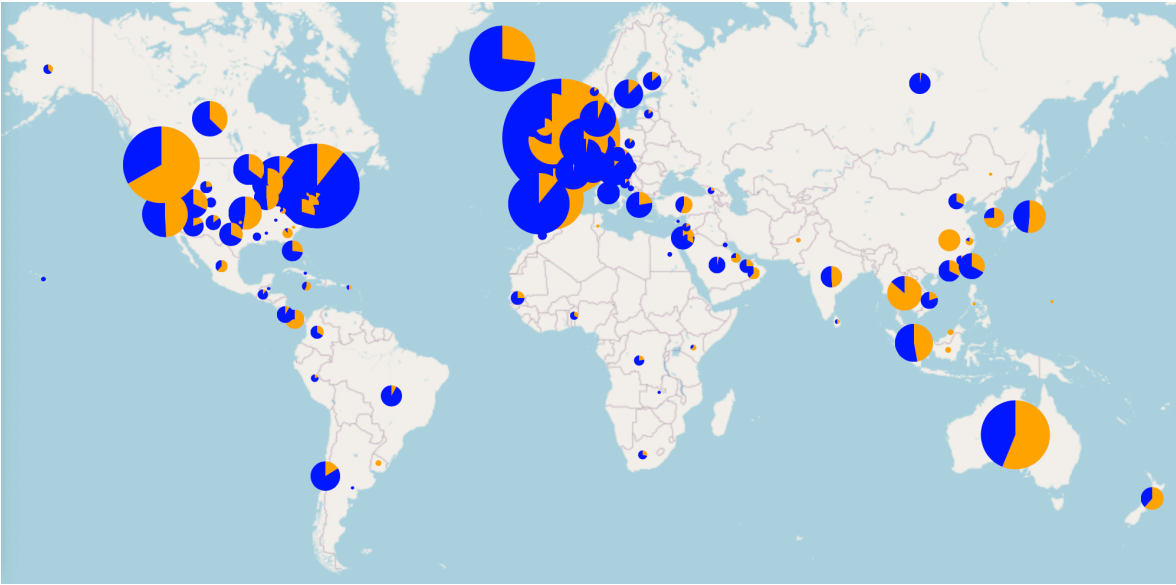
Sept 12, 2020: 76,467 Spike sequences

D614
G614

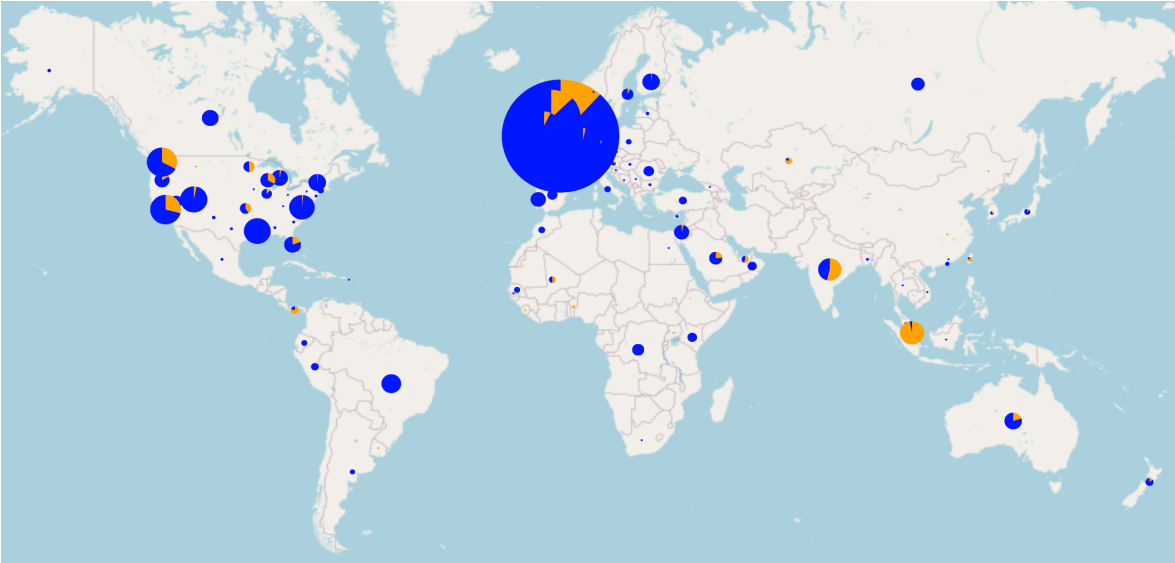
Prior to March 1



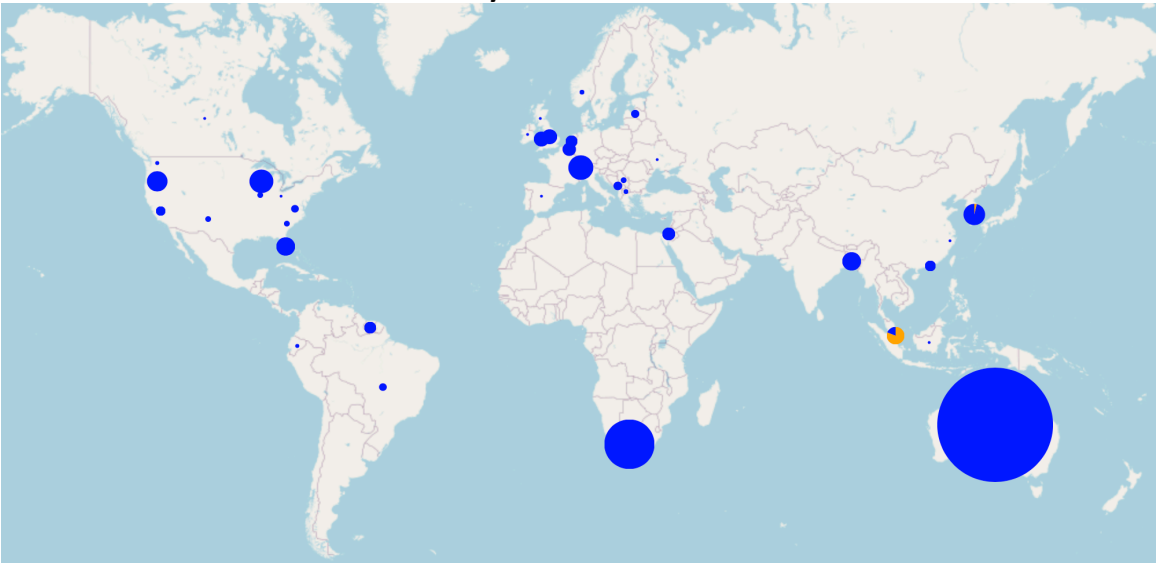
March 11-20

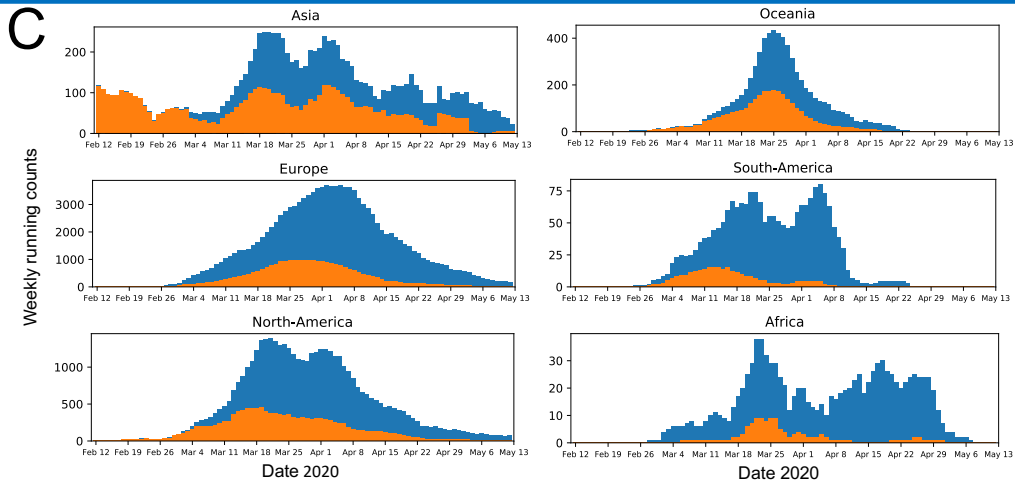
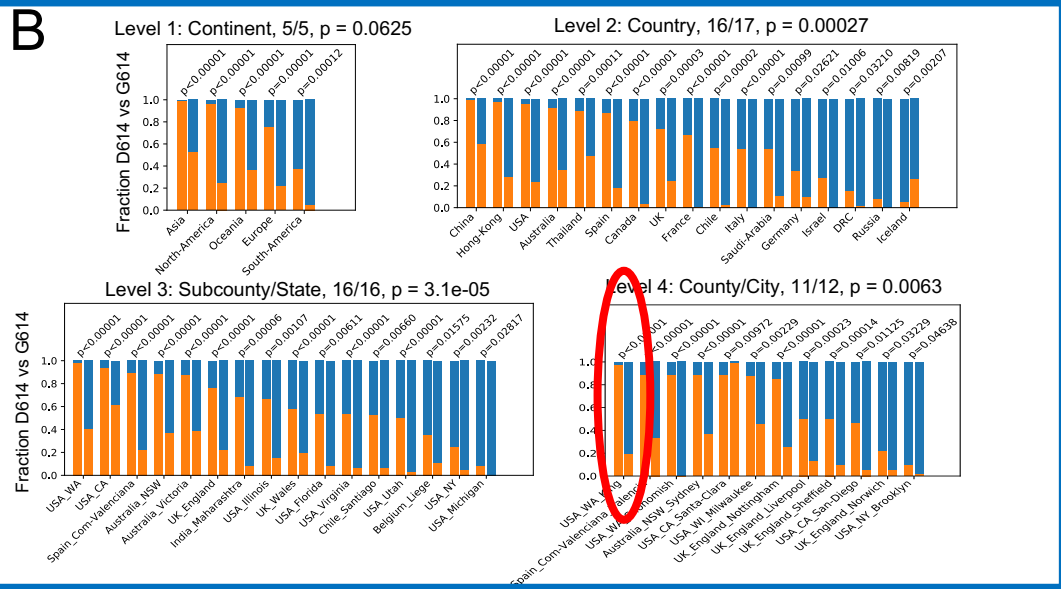
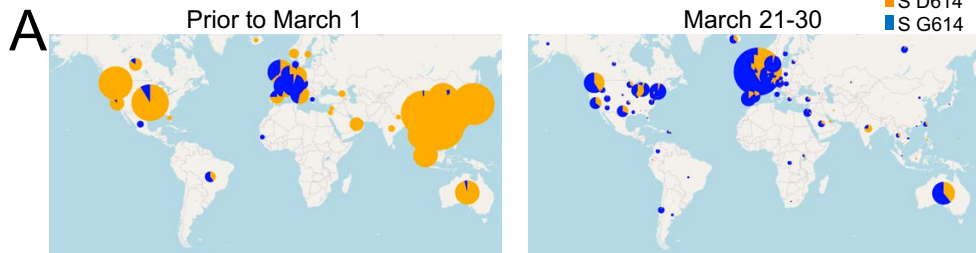


April 11-20



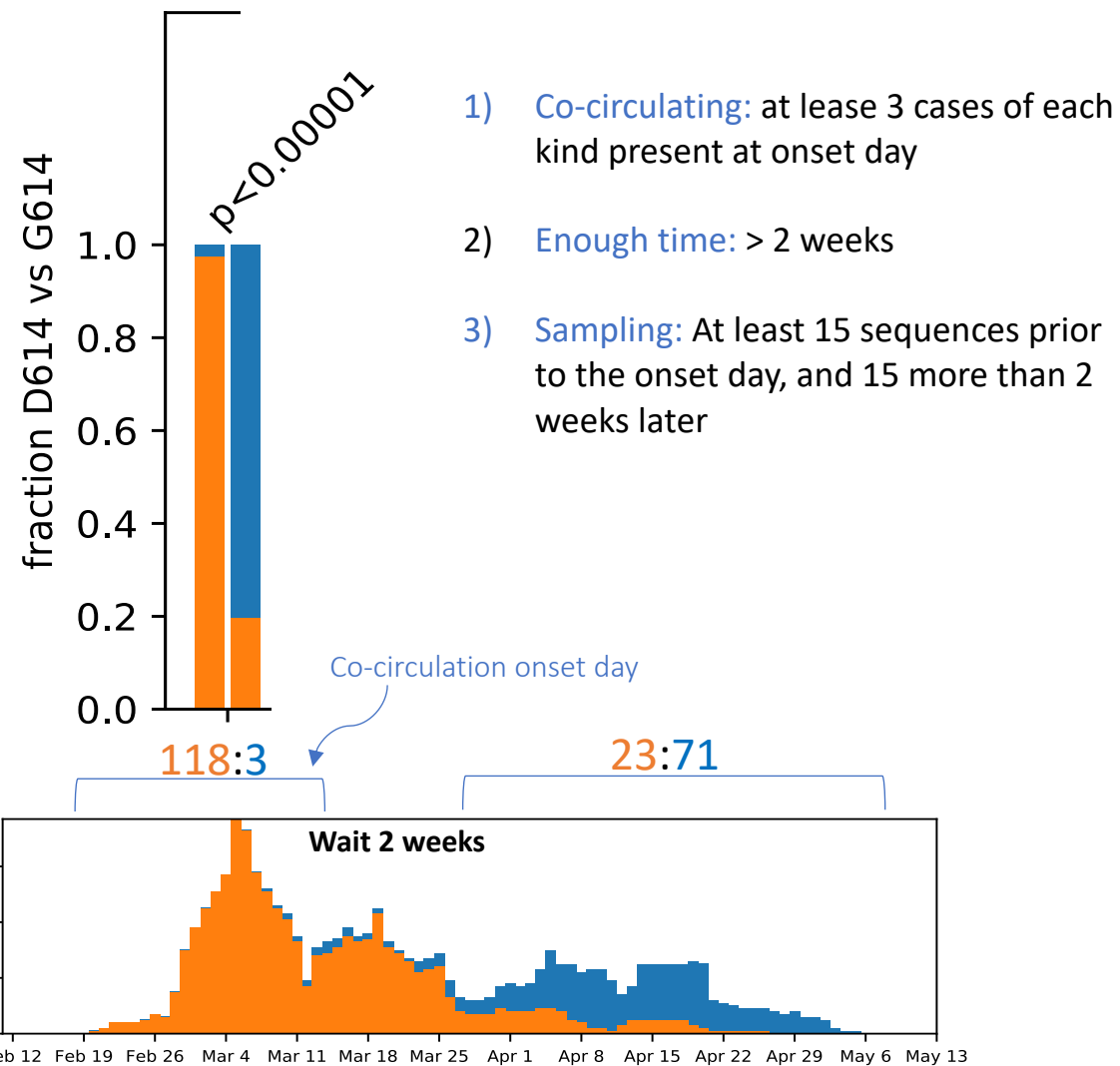
July 11-20





Systematically extract *all* GISAID regional data where: both forms were **co-circulating**, there was **enough time** to see a change, and there were enough sequences for statistical assessment

Washington King County

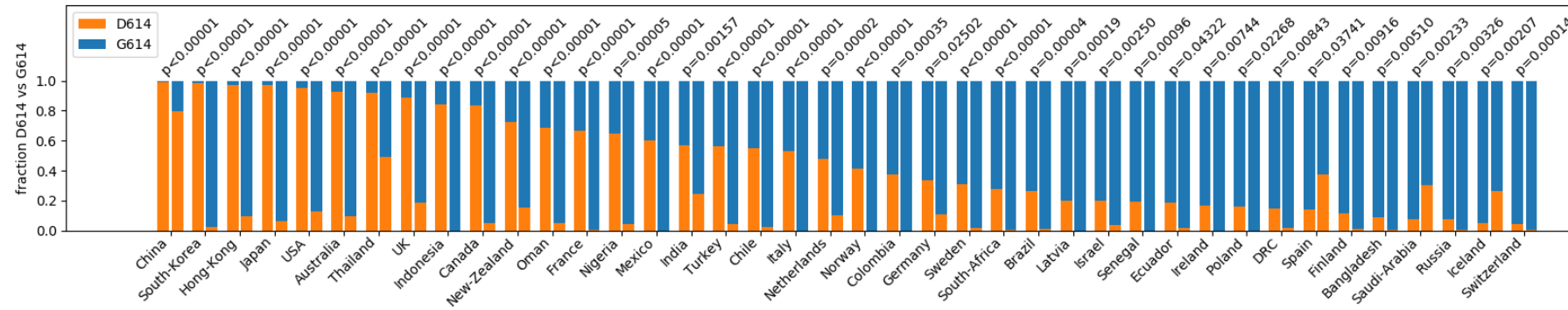


- 1) **Co-circulating**: at least 3 cases of each kind present at onset day
- 2) **Enough time**: > 2 weeks
- 3) **Sampling**: At least 15 sequences prior to the onset day, and 15 more than 2 weeks later

The GH and GR clades show no pattern of repeated increase globally: by country example:

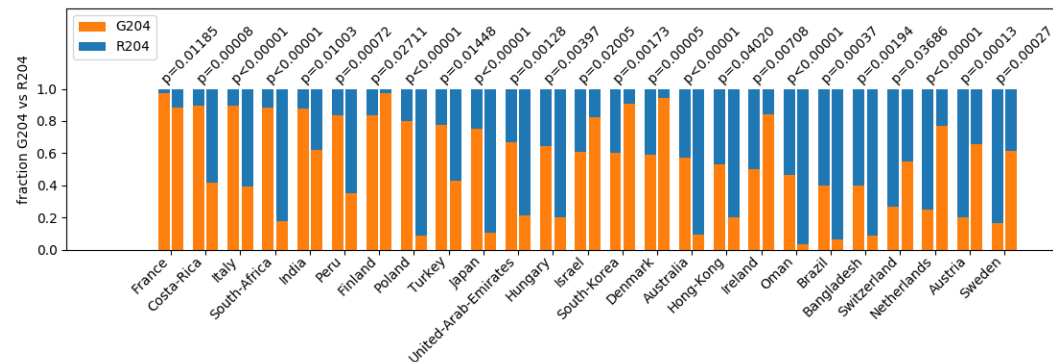
Data 9/12/2020

G



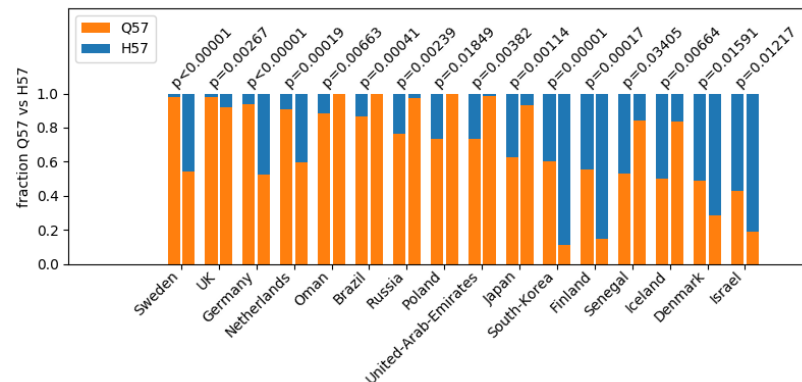
D614G is increasing
37/40
binomial
 $P = 1.9e-08$

GR, random shifts in frequency within the G clade:

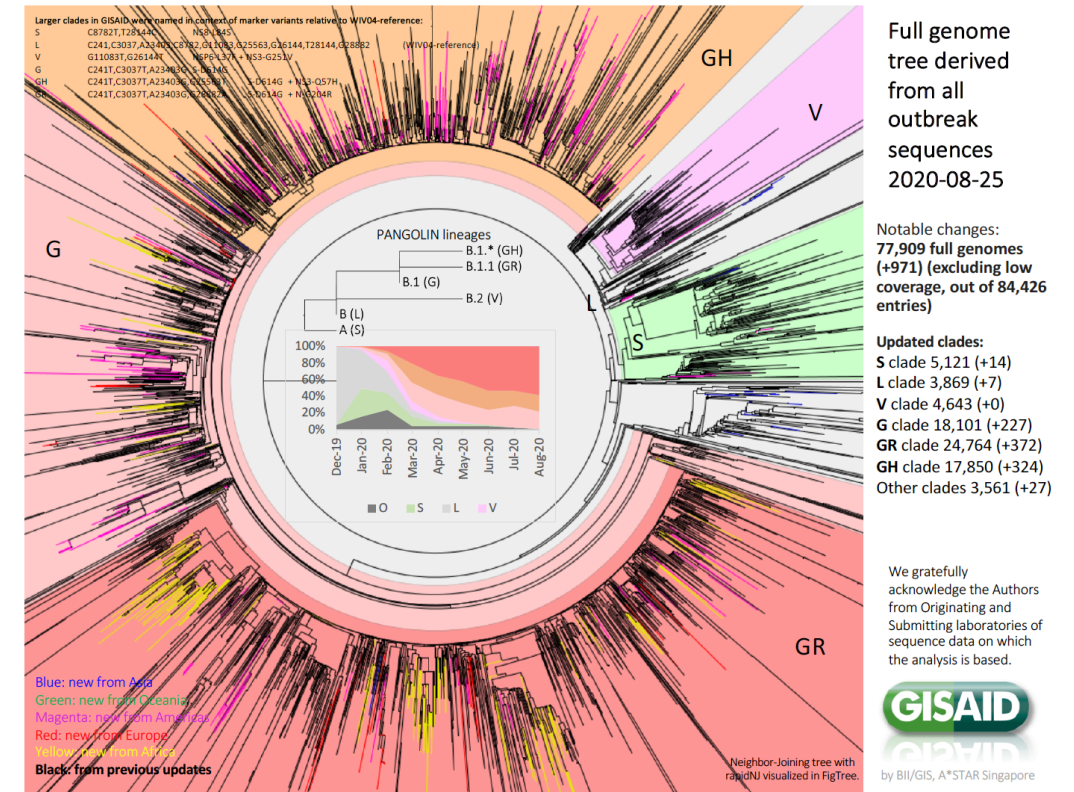


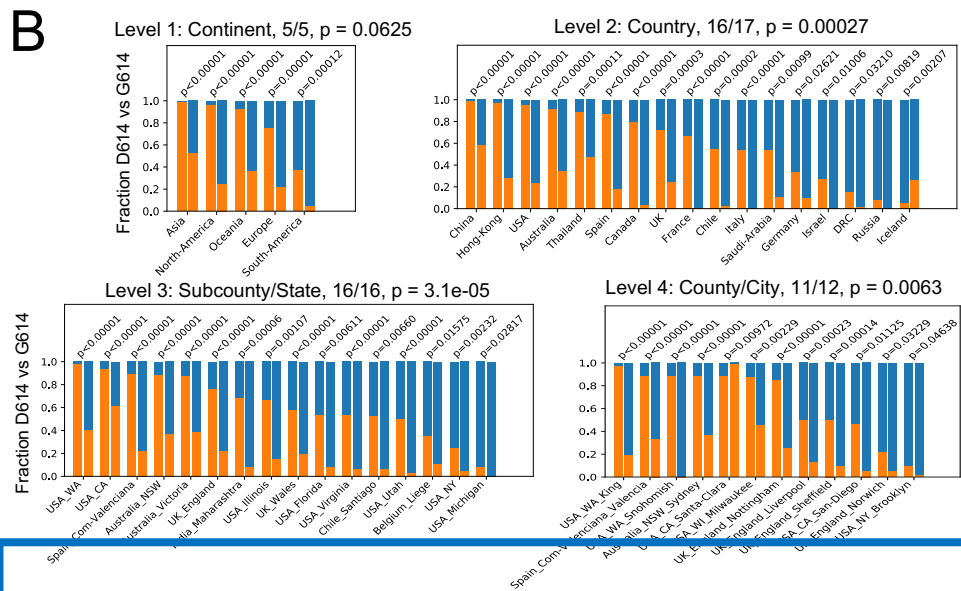
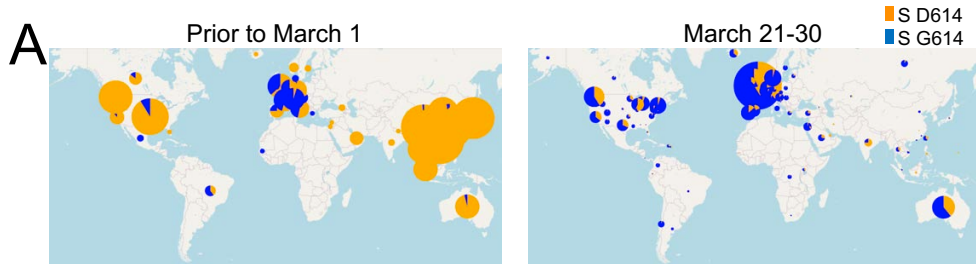
N G204R
Increasing
16/25
 $p = 0.23$

GH, random shifts in frequency within the G clade:



ORF 3a
Q57H
8/16
 $p = 1$



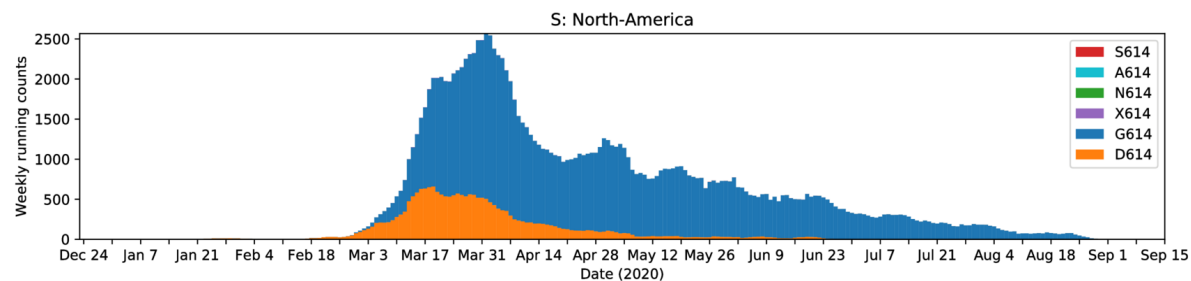
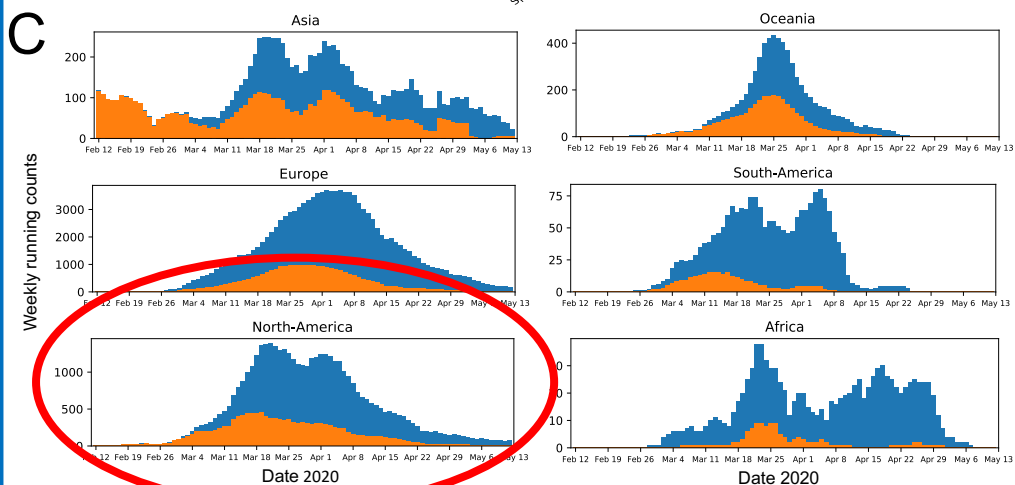
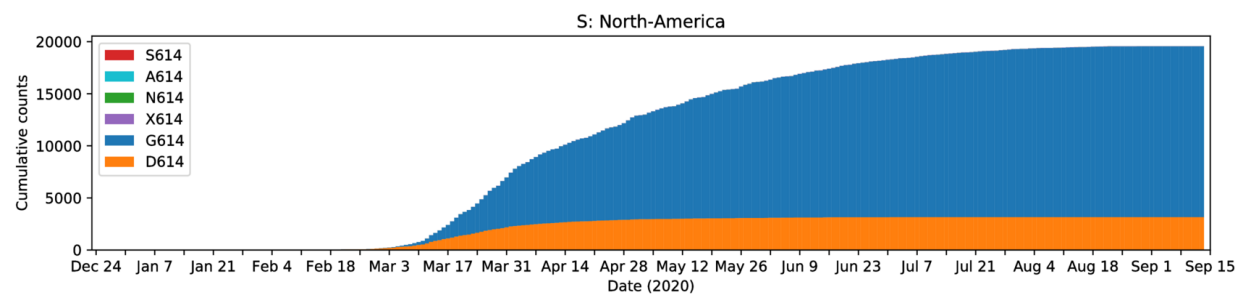


Focus on N American, 9/12/2020

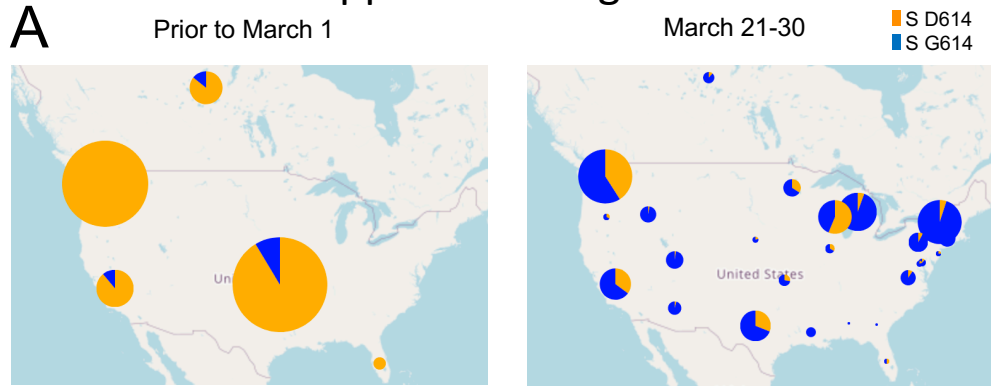
Cumulative, 19,563 good entries

D614: 3,150

G614: 16,390

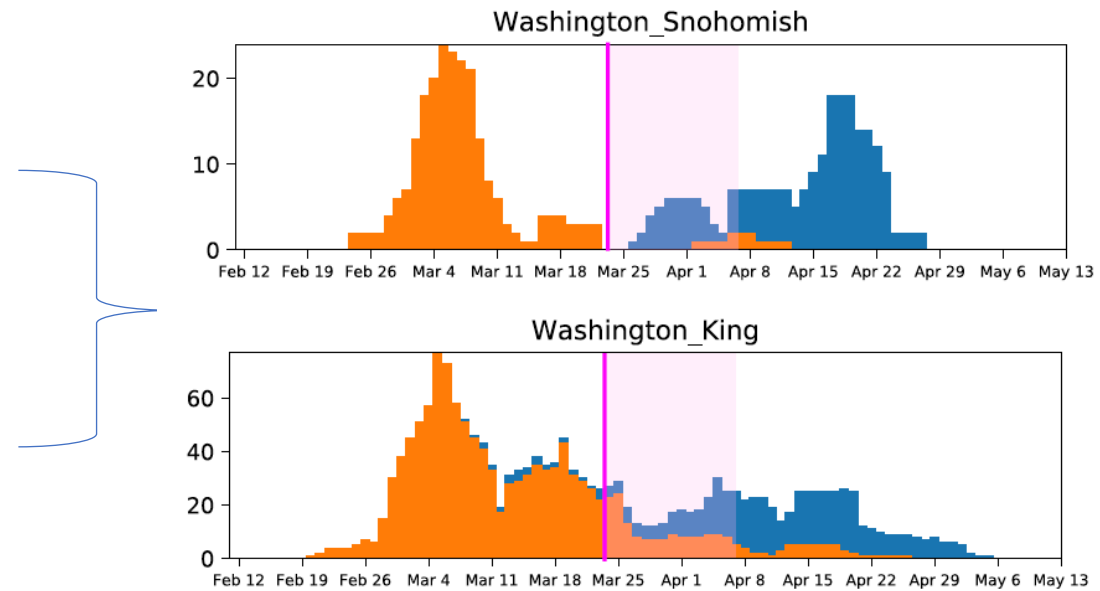
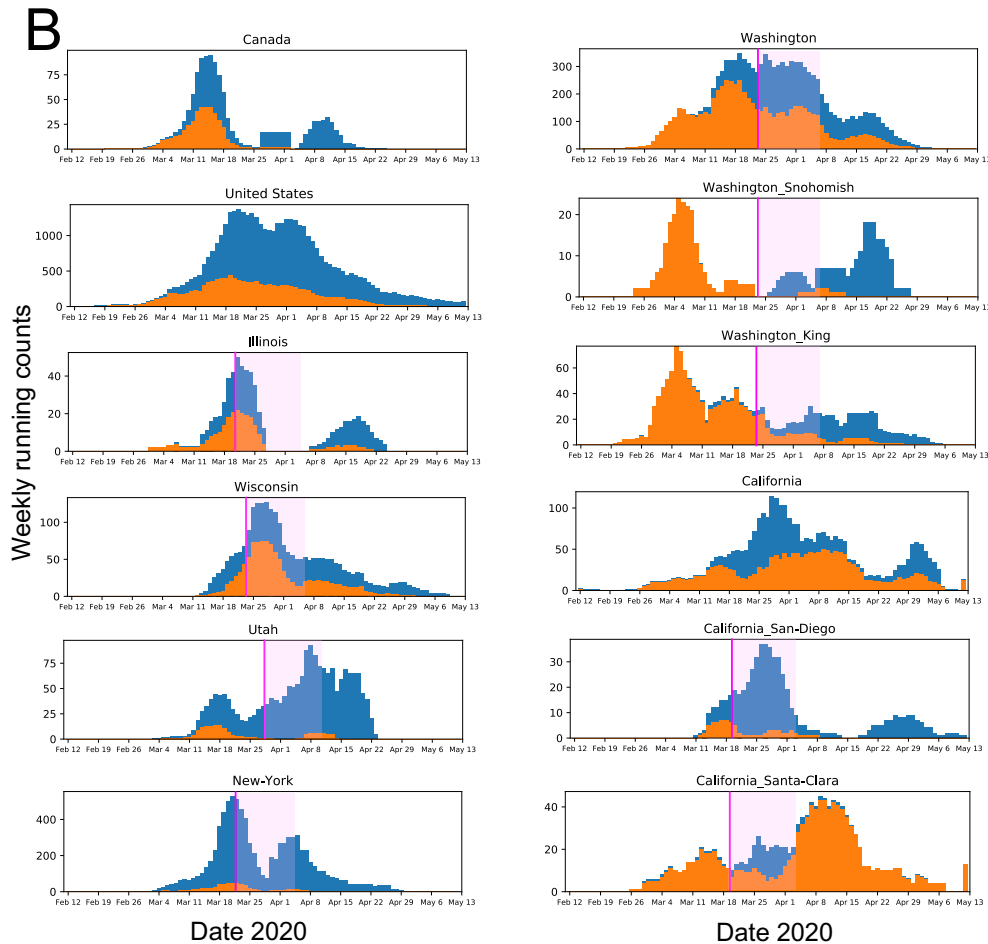


Supplemental figure 2



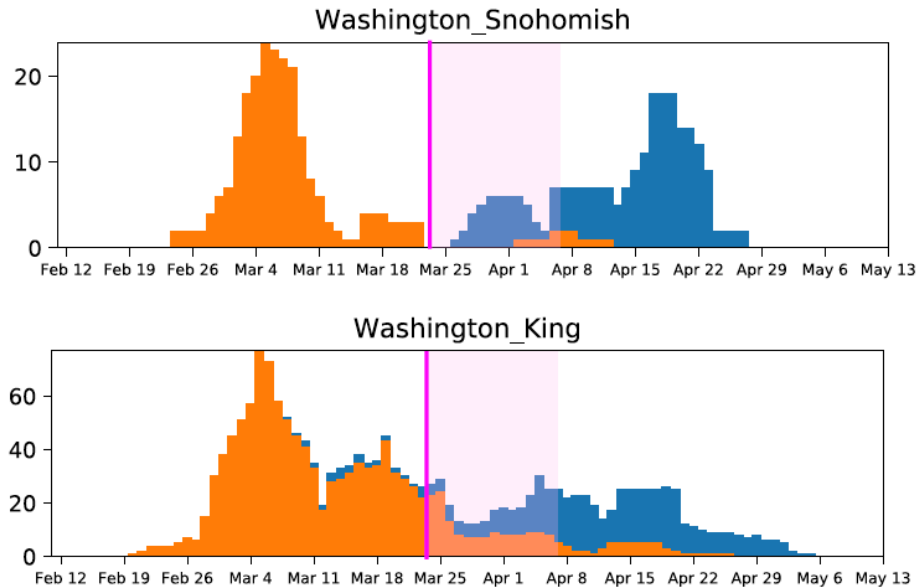
North America

The switch from D614 to G614 often happens even when G614 was introduced into very well established D614 epidemics



Break down...

Two counties in Washington State



On March 24th, Washington's stay-at-home orders were enacted:

County	Confirmed cases*	D614/N seqs	%D641
Snohomish	614	33/33	100%
King	1,170	153/161	95%

Number of infections per reported cases in Western WA: ~11.2 **

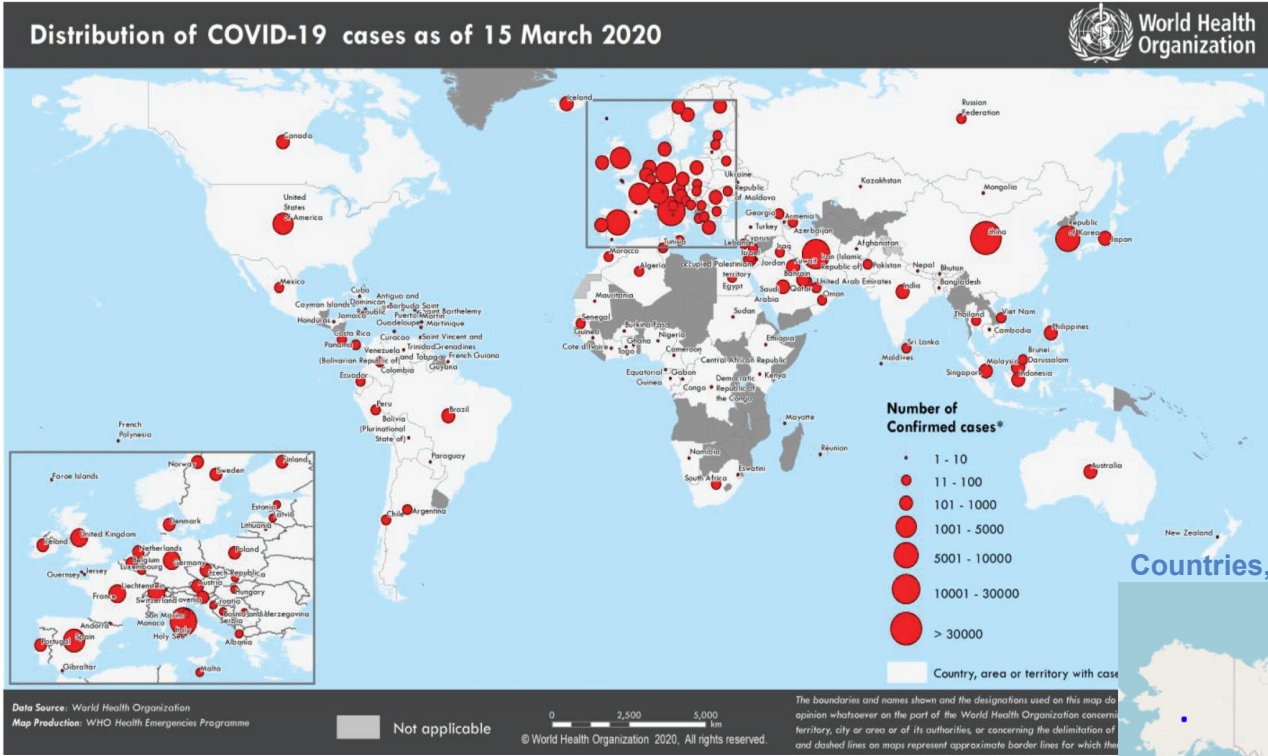
Thus, there were ~20,000 COVID infections in these two counties up through March 24, and >95% were D614

Could re-introductions from travelers during lock-down overwhelm a local epidemic of ~20,000 infections?

*From: COVID-19 Data Repository Johns Hopkins University

** Seroprevalence of Antibodies to SARS-CoV-2 in 10 Sites in the United States, March 23-May 12, 2020. Havers et al. JAMA July online ahead of print July 21.

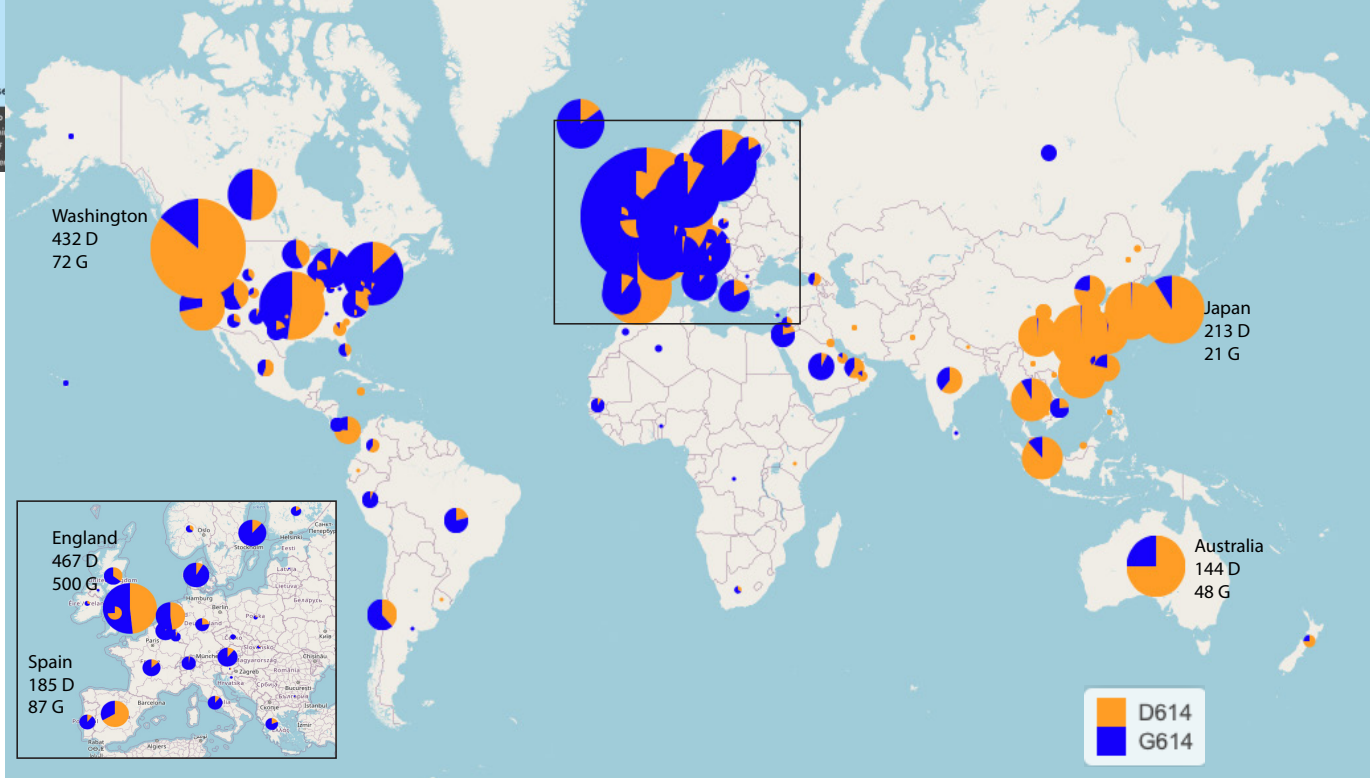
Figure 1. Countries, territories or areas with reported confirmed cases of COVID-19, 15 March 2020



← WHO map of confirmed cases as of March 15

cov.lanl.gov map of D614/G614 as of March 15

Countries, territories or areas with SARS-CoV-2 sequences in GISAID, D/G distribution, 03/15/2020



It was not just about travelers from China and Europe

There were many very well established predominantly D clade epidemics, all over the world:

- Western USA,
- Australia,
- Europe: Wales, Spain
- All over Asia

A different view: isotonic logistic regression: The “city” level example from Fig 3,

All geographic samples (country, state, county, city) were extracted from GISAID that met the following criteria:

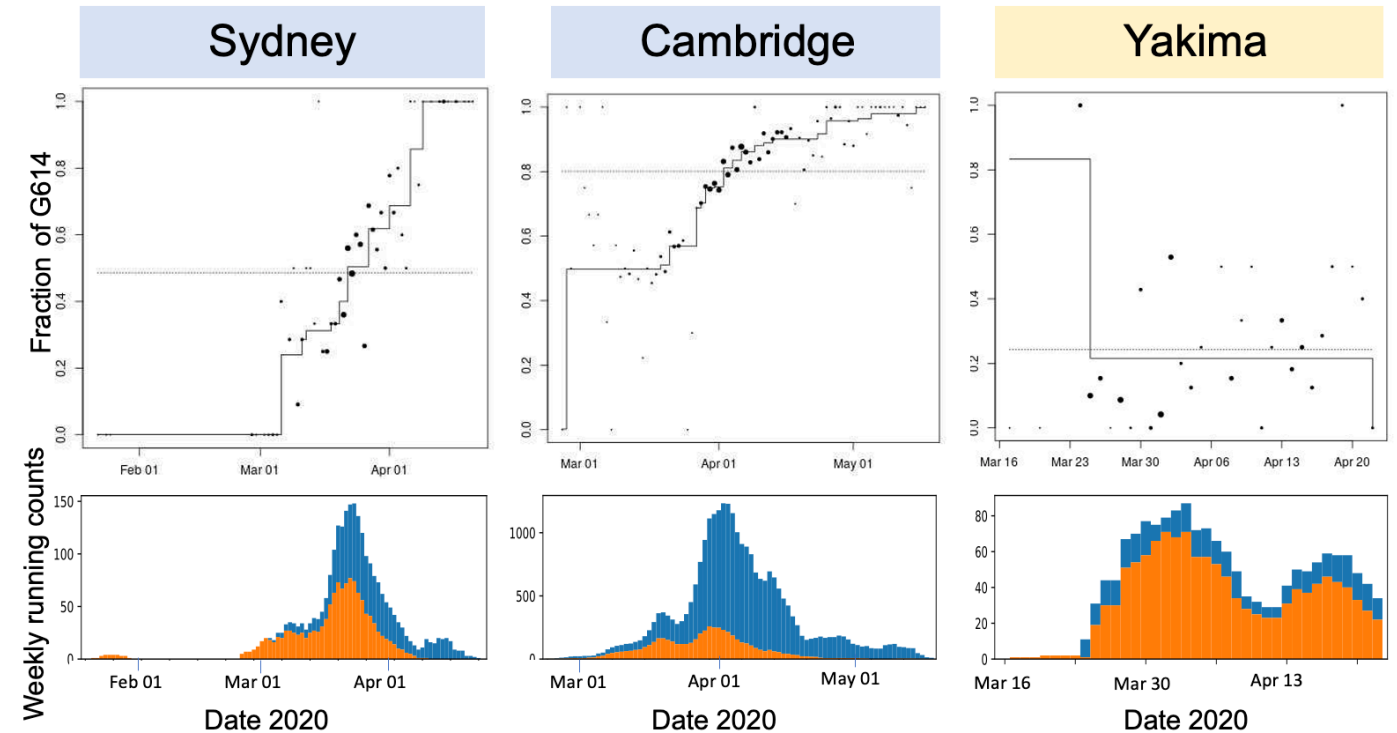
5 sequences representing each of the D614 and the G614 variants, and least 14 days sampled.

We tested the null against 2 hypotheses: that the fraction of G614 either increases, or that it decreases

We permuted the data 4000 times, refitting the isotonic logistic regression, to estimate the p-values

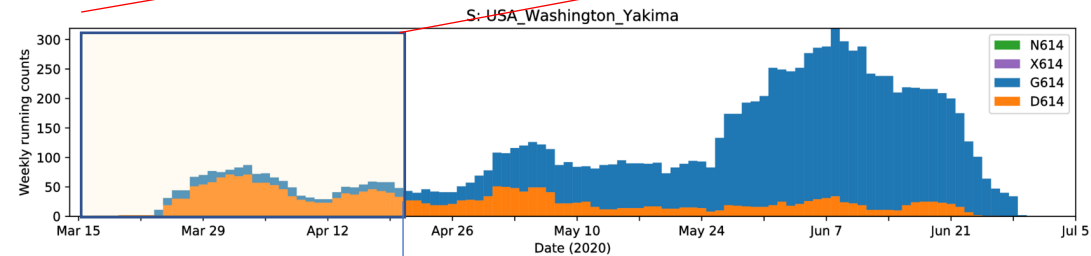
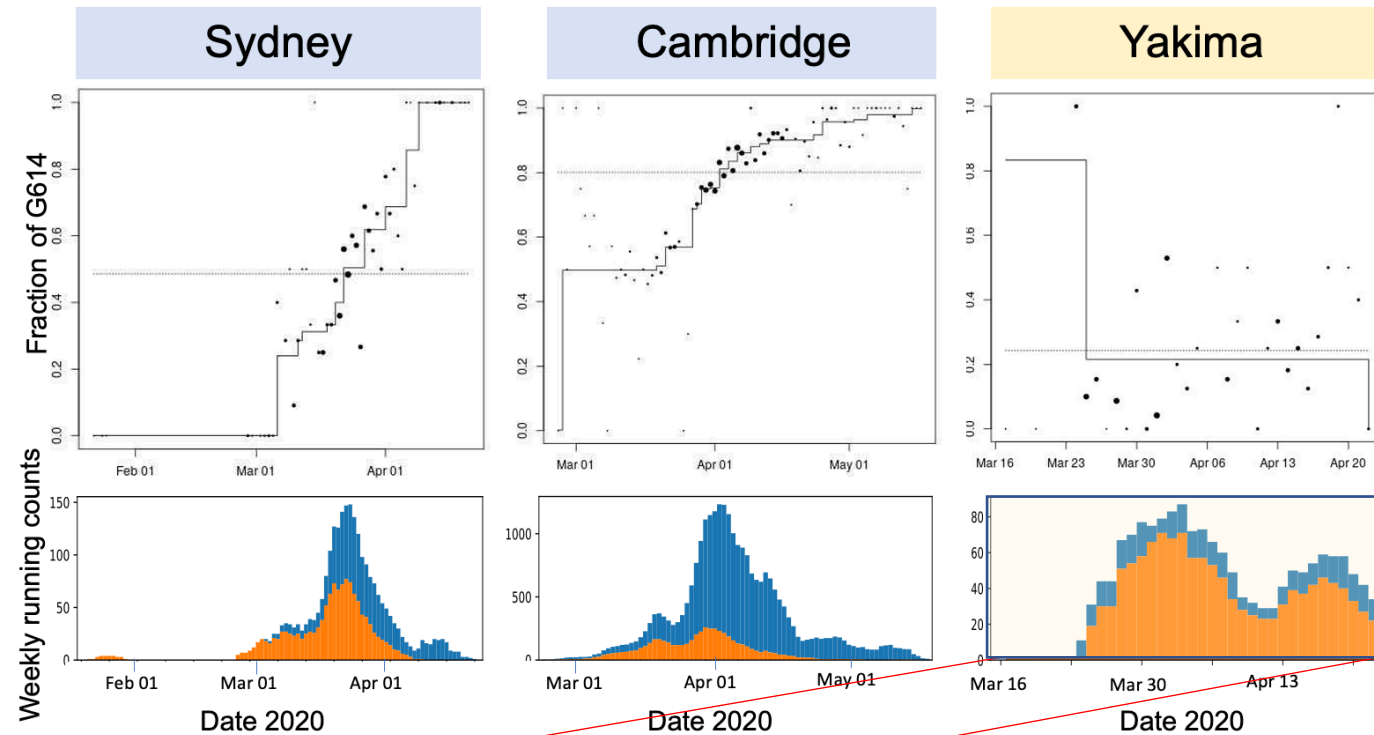
The “city” level example from Fig 3:

19 cities with a clear direction, 17 increasing: p = 0.0007						
Level 3: County/City	# D614	# G614	# of days	Time window days	G614 increasing p-value	G614 decreasing p-value
Australia_New-South-Wales_Sydney	189	179	51	90	0.00025	1.00
Spain_Comunitat-Valenciana_Valencia	72	97	30	34	0.00025	0.64
United-Kingdom_England_Bristol	240	629	35	37	0.00025	0.28
United-Kingdom_England_Cambridge	751	3020	81	81	0.00025	1.00
United-Kingdom_England_Liverpool	97	484	46	45	0.00025	0.71
United-Kingdom_England_Nottingham	204	386	67	76	0.00025	0.99
United-Kingdom_England_Sheffield	120	431	44	51	0.00025	1.00
USA_Washington_King	173	75	58	69	0.00025	0.99
USA_Washington_Pierce	32	35	21	38	0.00025	1.00
USA_Washington_Snohomish	35	32	27	93	0.00025	1.00
USA_Wisconsin_Milwaukee	66	30	32	45	0.00025	0.97
United-Kingdom_England_Norwich	29	269	26	28	0.00075	0.97
USA_California_San-Diego	11	75	33	58	0.002	0.95
United-Kingdom_England_London	36	357	19	24	0.0085	0.91
USA_Wisconsin_Madison	13	43	26	35	0.030	0.39
USA_New-York_Manhattan	38	339	30	45	0.036	0.90
USA_California_San-Francisco	59	83	21	48	0.049	0.34
USA_New-York_Brooklyn	13	292	31	46	0.070	0.87
USA_Washington_Yakima	184	59	31	36	0.073	0.00025
USA_California_Santa-Clara	165	24	50	76	0.49	0.00025



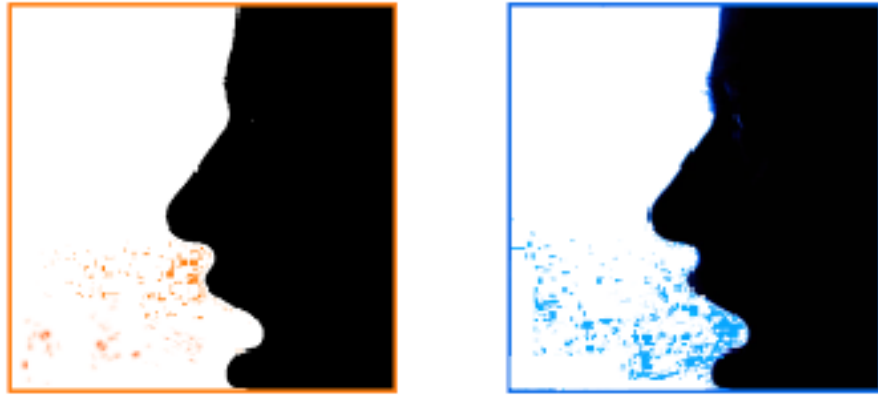
Yakima just need more time to shift, and is no longer an exception

The *current* Yakima data shows the shift towards G614 as significant: $p = 0.00025$ -> $p = 0.00025$



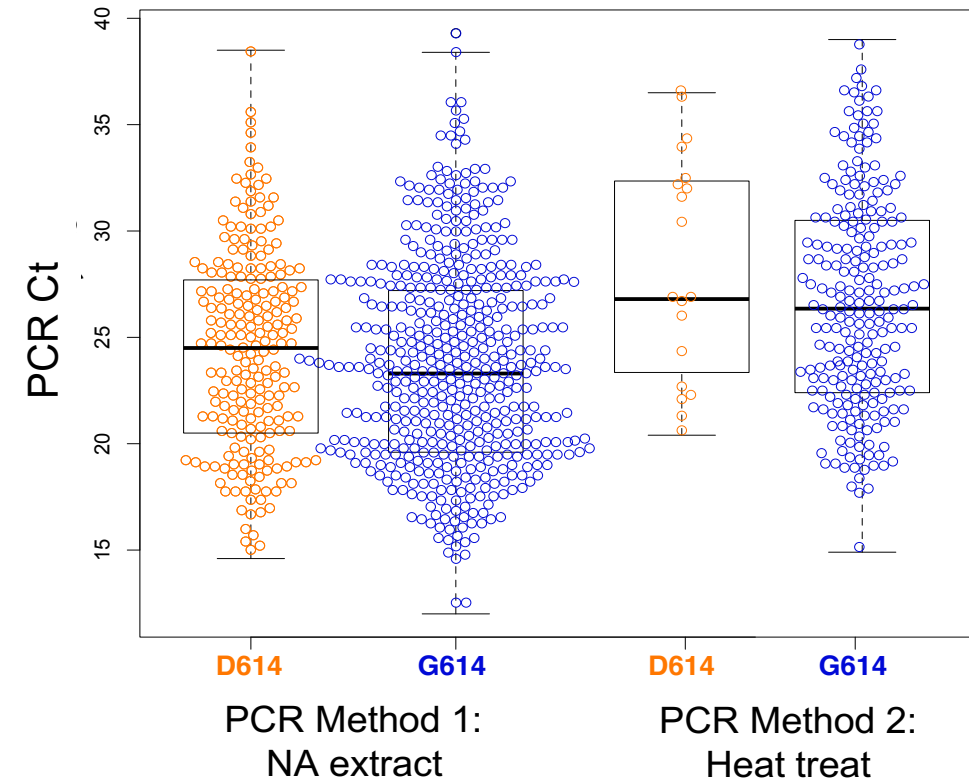
Cell paper 05/29/2020 243 sequences spanning 36 days	Recent 09/12/20 1620 sequences spanning 100 days
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G614 is associated with higher viral loads in patients indicated by lower RT PCR cycle thresholds for detection



Ct **D614** vs **G614**
GLM $p = 0.037$

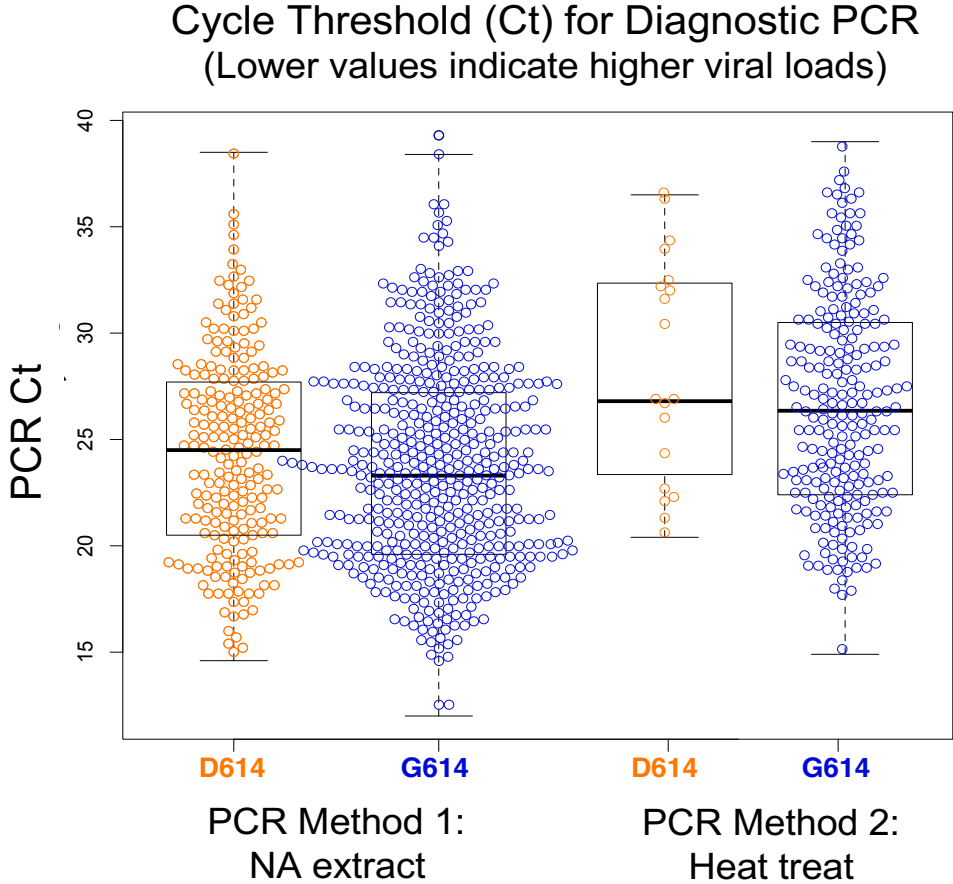
Cycle Threshold (Ct) for Diagnostic PCR
(Lower values indicate higher viral loads)



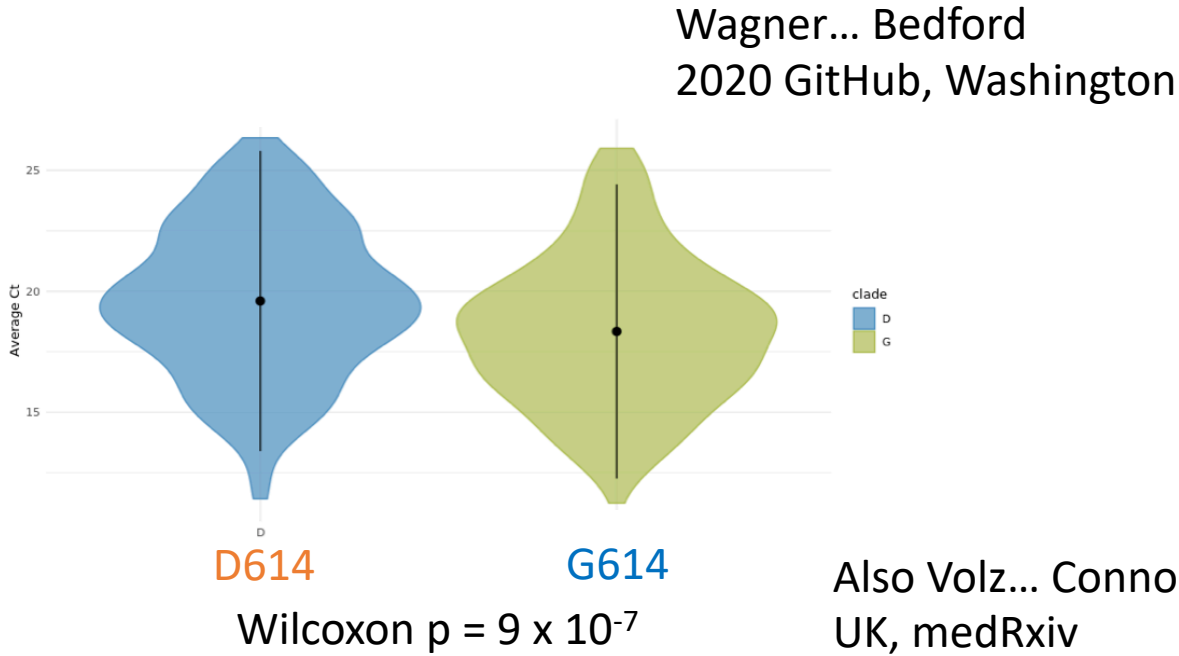
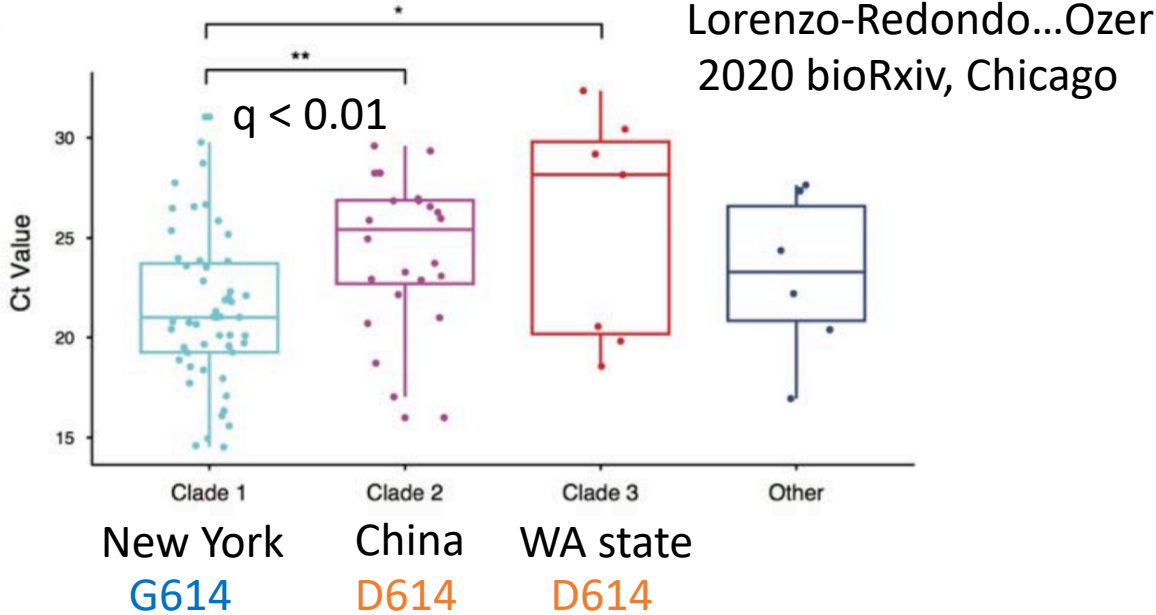
~1000 Thushan de Silva, Sheffield, England (Fig. 5)

Our findings are supported in three additional preprints

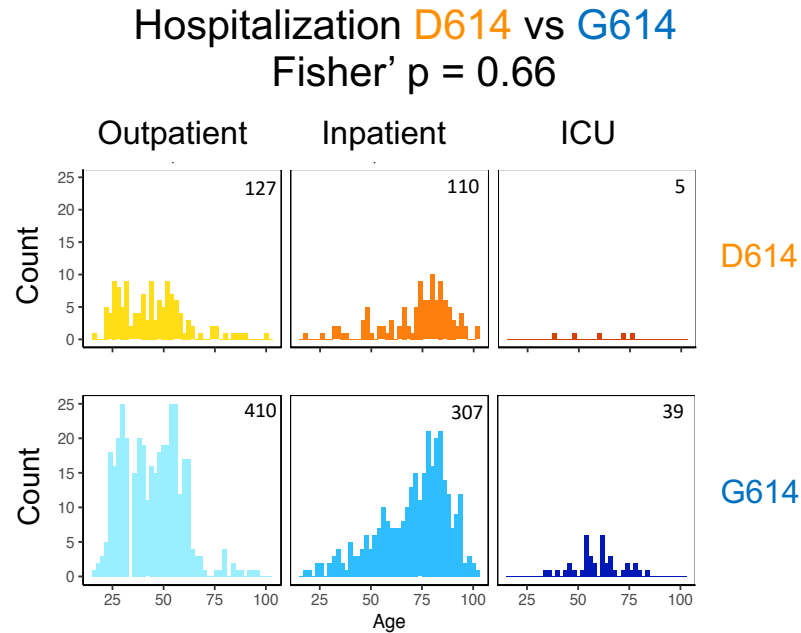
Ct D614 vs G614
GLM $p = 0.037$



~1000 patients, Thushan de Silva, Sheffield, England (Fig. 5)



No Association was found between D614 and G614 status and hospitalization



	OP	IP	ICU
D614	127	110	5
G614	410	307	39

Fisher's exact, 2x2: (OP+IP) x ICU = 0.047
Fisher's exact, 2x2, OP x (IP+ICU): p = 0.66

The three preprints that confirmed the association between lower Ct and G614 status also found no increase in virulence associated with G614

Hospitalization was not increased

Lorenzo-Redondo...Ozer
2020 bioRxiv, Chicago

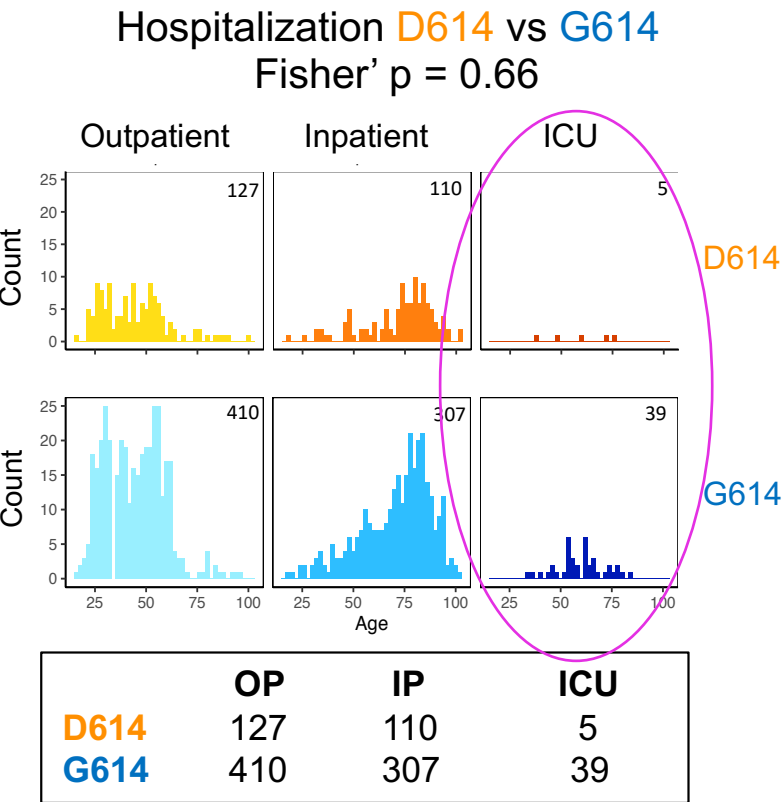
Wagner... Bedford
2020 GitHub, Washington

Mortality was not increased

Volz... Connor
2020 medRxiv, UK

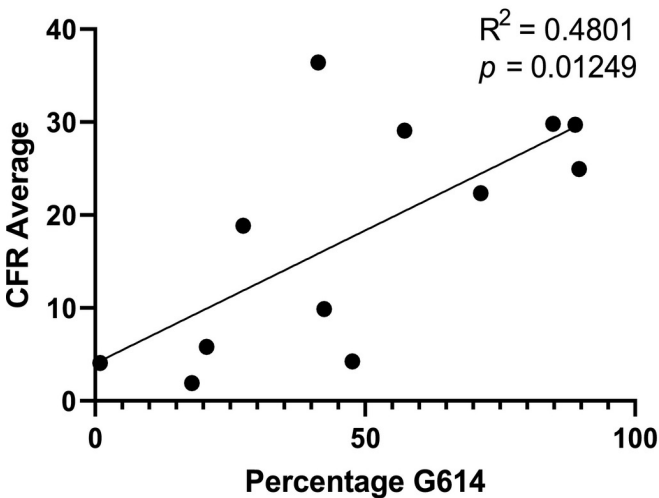
But hospitalization was highly correlated with age and being male

Although no Association was found between D614 and G614 status and hospitalization



Fisher's exact, 2x2: (OP+IP) x ICU = 0.047
Fisher's exact, 2x2, OP x (IP+ICU): p = 0.66

But... we found a trend between G614 status and ICU, and Beccera-Flores report an association with fatality rates at the country level



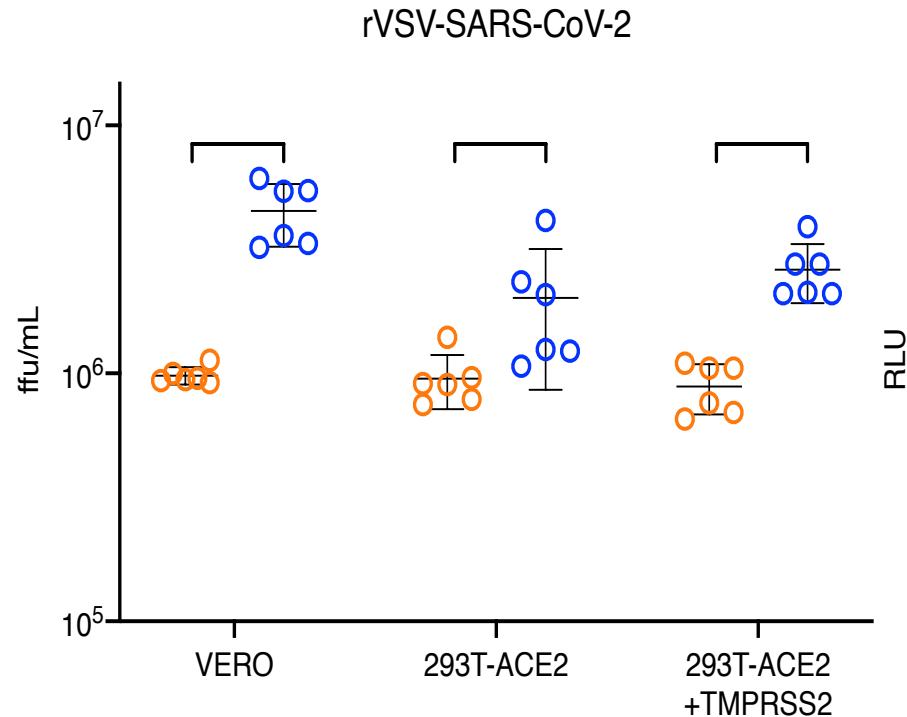
Data sampled April 6, 2020

Country	Percentage G614 Mutation	CFR Average
Belgium	88.89	29.73
France	84.82	29.83
China	0.89	4.07
Germany	47.62	4.25
Netherlands	57.30	29.07
Brazil	71.43	22.36
Canada	42.40	9.89
Italy	89.66	24.93
Australia	17.91	1.92
Japan	20.62	5.82
Spain	41.30	36.43
US	27.42	18.85

Beccera-Flores & Cardozo, Int J Clin Pract, e13525 (2020)

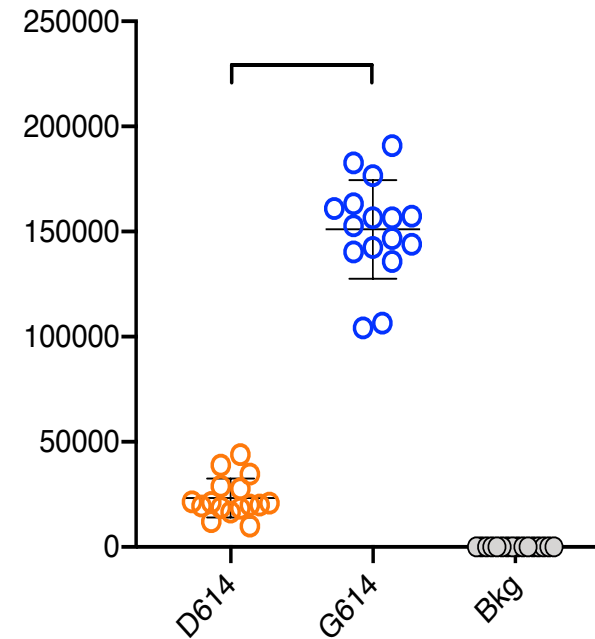
The **G614** Spike is more infectious in a pseudotype viral assays than the **D614** Spike

rVSV, Erica Ollmann Safire
Vesicular Stomatitis Virus

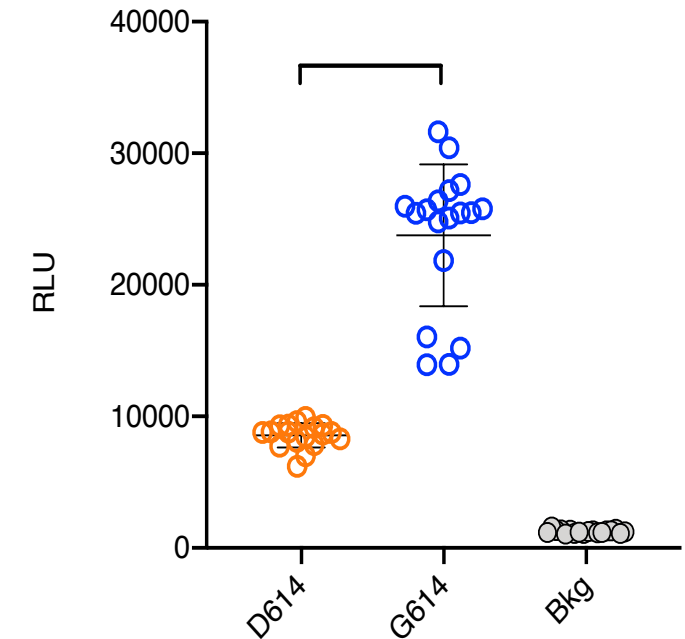


Lentiviral particles, David Montefiori

SARS-CoV-2pp, 293T/ACE2



SARS-CoV-2pp, TZM-bl/ACE2



Also independently shown by:

Zhang... Farzan & Choe, BioRxiv

The D614G mutation in the SARS-CoV-2 spike protein reduces S1 shedding and increases infectivity

Yurkovetskiy... Luban, BioRxiv...

Structural and Functional Analysis of the D614G SARS-CoV-2 Spike Protein Variant

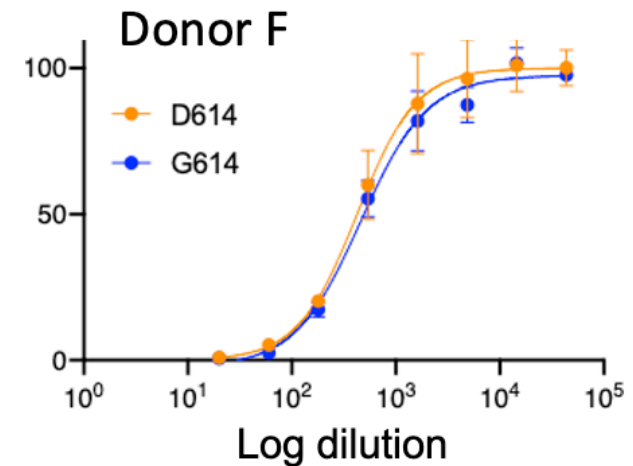
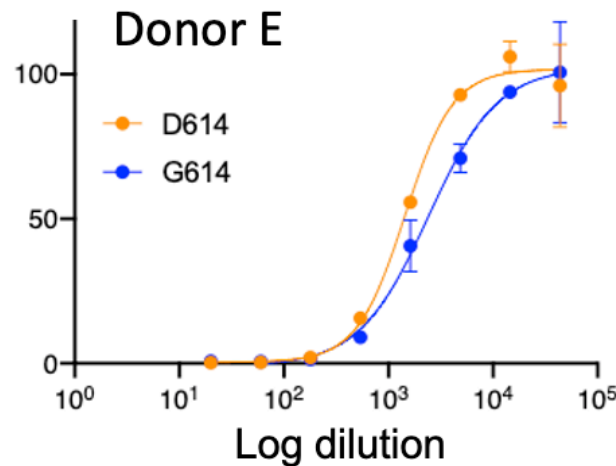
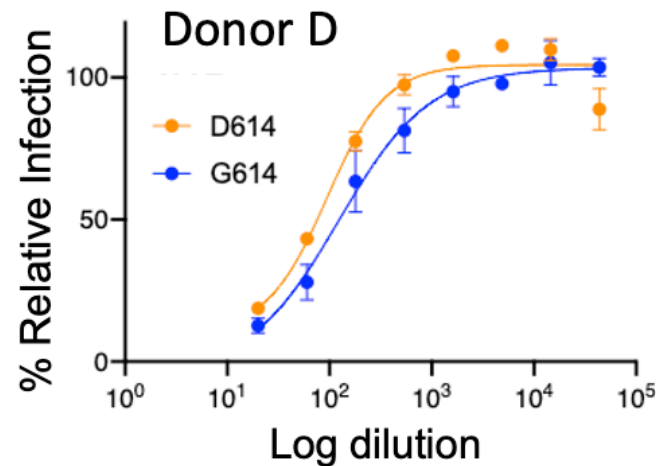
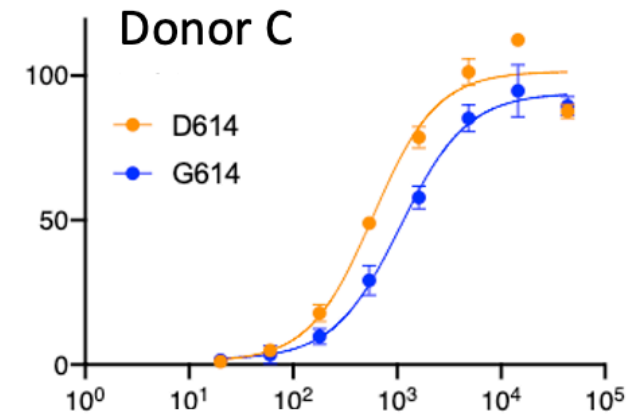
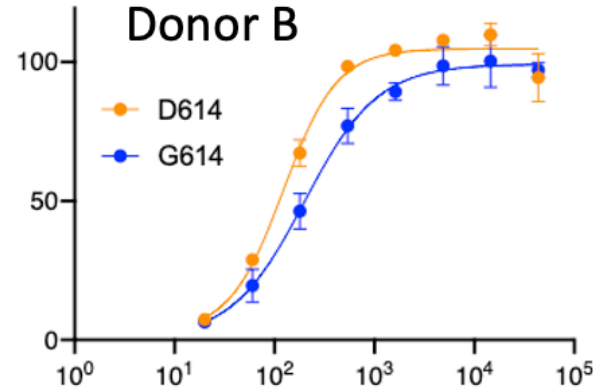
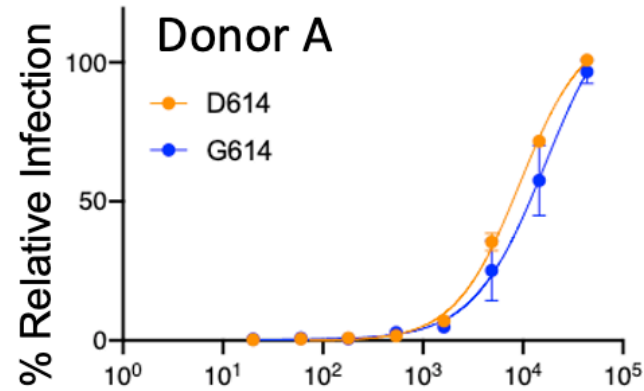
Pseudotype virus neutralization measurements are highly correlated with authentic SARS-CoV-2 measurements:

Schmidt et al. ... Bieniasz. J Exp Med 2020 Nov 2;217(11):e20201181.

Measuring SARS-CoV-2 neutralizing antibody activity using pseudotyped and chimeric viruses

The **G614** Spike is at least as as neutralization sensitive as **D614** to polyclonal Abs in COVID-19 Convalescent Sera

The D/G status of the infecting strain was not known.



The **D614G** Spike Mutation *Increases* SARS CoV-2 Susceptibility to Neutralization

Drew Weissman, Mohamad-Gabriel Alameh, Thushan de Silva, Paul Collini, Hailey Hornsby, Rebecca Brown, Celia C. LaBranche, Robert J Edwards, Laura Sutherland, Sampa Santra, Katayoun Mansouri, **Sophie Gobeil**, Charlene McDanal, Norbert Pardi, Nick Hengartner, COVID-19 Genomics Consortium UK, Paulo J.C. Lin, Ying Tam, Pamela A. Shaw, Mark G. Lewis, Carsten Boesler, Uğur Şahin, **Priyamvada Acharya**, Barton F. Haynes, Bette Korber, **David C. Montefiori**

MEDRXIV/2020/159905

<https://www.medrxiv.org/content/10.1101/2020.07.22.20159905v1>

G614 Spike is more sensitive than D614 to vaccine sera from mice, NHP and human

Immunized with the nucleoside-modified mRNA-LNP vaccine platform

Four different variants of the Spike immunogen:

- 1 monomeric secreted RBD
- 2 trimeric secreted RBD
- 3 diProline stabilized D614 Spike
- 4 Furin mutant D614 Spike, S1 and S2 subunit associations maintained

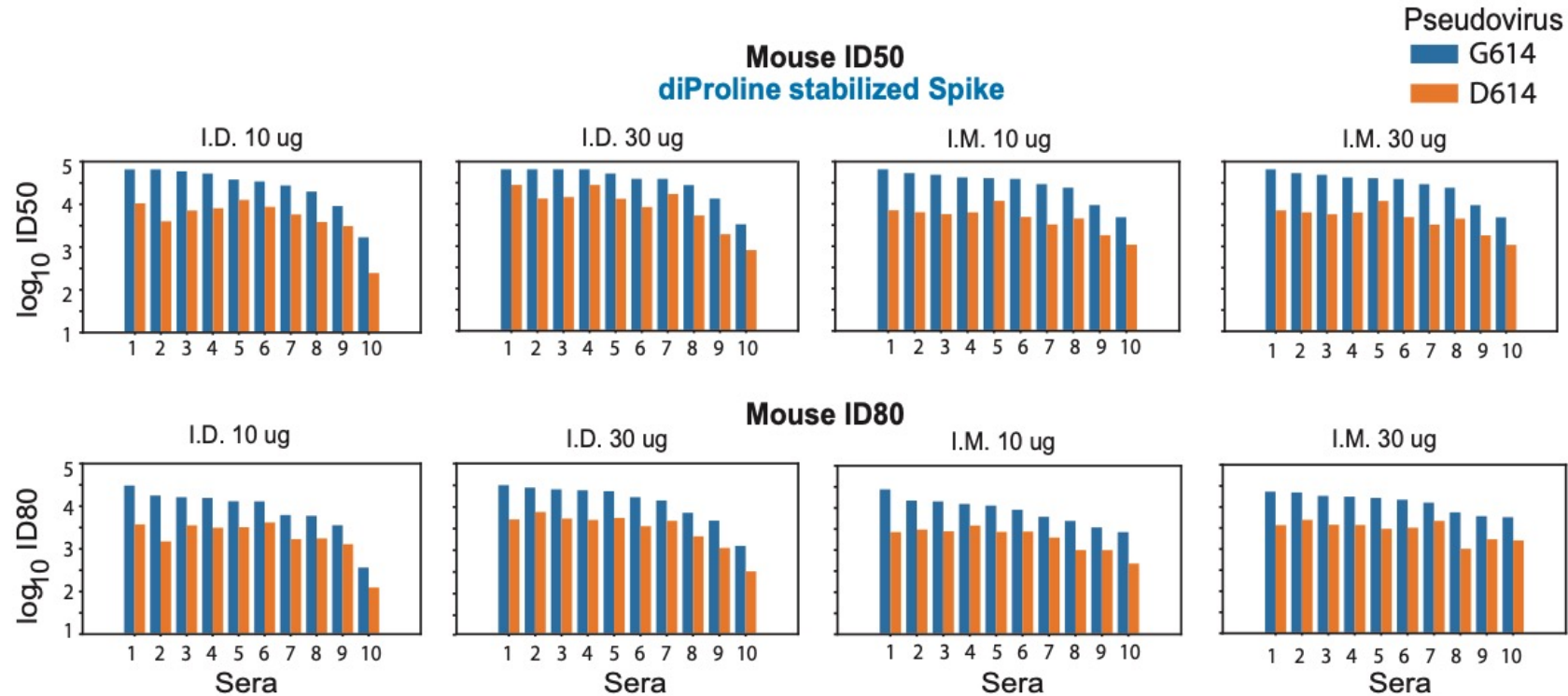
Pseudovirus and neutralization assay:

SARS-CoV-2 neutralization was assessed with Spike-pseudotyped viruses in 293T/ACE2 cells as a function of reductions in luciferase (Luc) reporter activity.

Spike D614 and G614 pseudotype viruses were created in a lentivirus backbone.

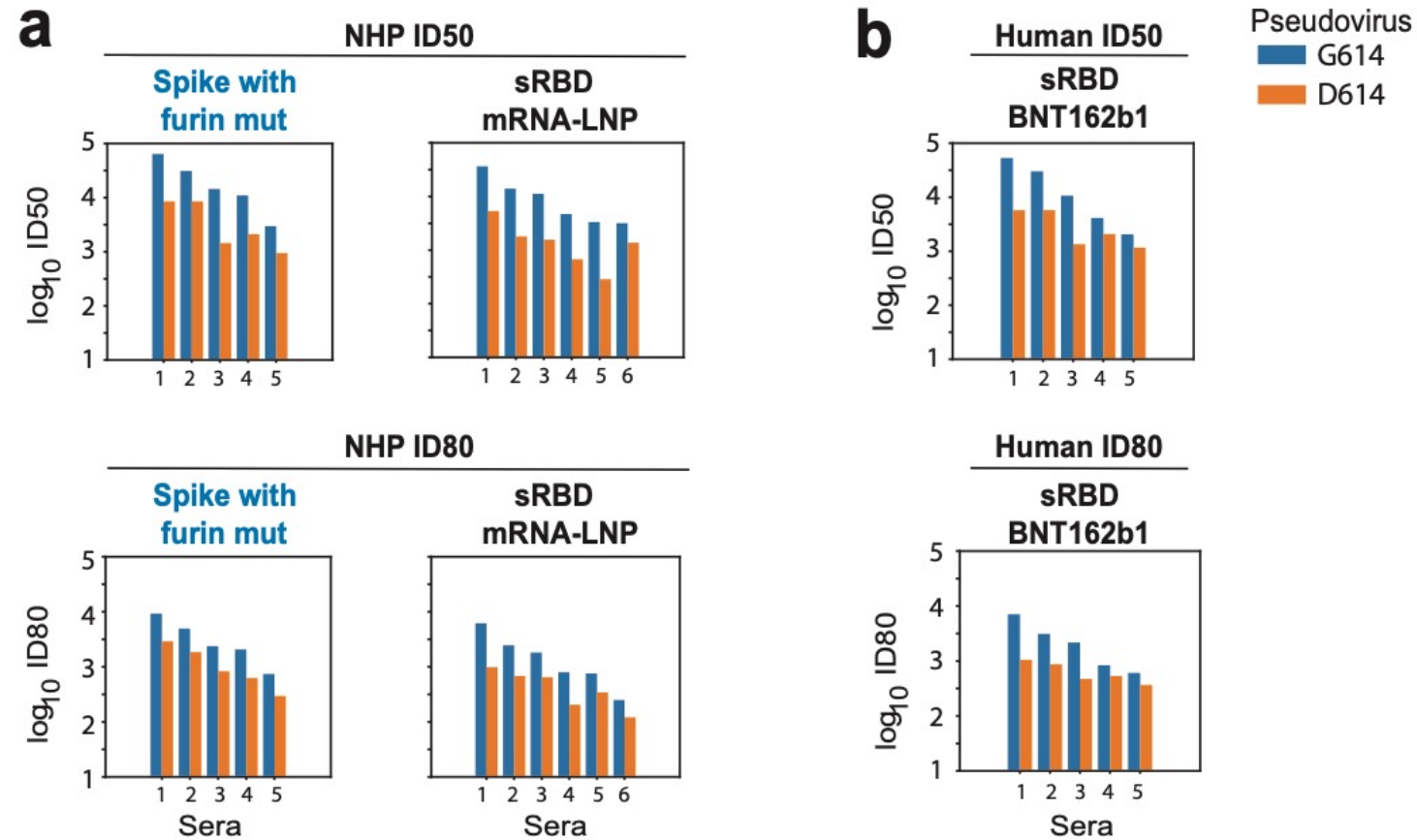
4 groups of 10 mice each, comparing dose and delivery

The sera from vaccinated mice were evaluated for neutralization potency, comparing D614 and G614 pseudoviruses.
Each pair of bar graphs represents one serum, G614 is always more sensitive



Route	Dose (μ g)	ID50 Geometric mean G:D ratio, 95% CI	p-value	ID80 Geometric mean G:D ratio, (95% CI)	p-value
I.D.	10	5.6 (3.9 — 8.1)	2.0e-06	4.4 (3.2 — 6.2)	3.6e-06
I.D.	30	3.9 (2.9 — 5.1)	1.5e-06	4.3 (3.7 — 5.0)	3.8e-09
I.M.	10	6.5 (5.1 — 8.3)	3.3e-08	4.5 (3.5 — 5.8)	3.5e-07
I.M.	30	5.2 (3.8 — 7.0)	7.8e-07	4.6 (3.7 — 5.6)	4.8e-08
All 40 mice		5.2 (4.5 — 6.0)	2.2e-16	4.4 (4.0 — 4.9)	2.2e-16

G614 enhanced sensitivity also seen in Non-Human Primates (NHPs) and People



Species	Vaccine	ID50 Geometric mean G:D ratio, 95% CI	p-value	ID80 Geometric mean G:D ratio, (95% CI)	p-value
NHP	Spike furin mut	5.4 (3.0 — 9.8)	0.0015	2.9 (2.5 — 3.3)	<0.001
NHP	sRBD mRNA-LNP	6.5 (3.7 — 11.4)	<0.001	3.2 (2.1 — 5.0)	<0.001
Human	sRBD BNT162b1	4.2 (1.6 — 11.0)	0.014	3.1 (1.4 — 6.8)	0.017

G614 Spike is more sensitive to convalescent sera than D614

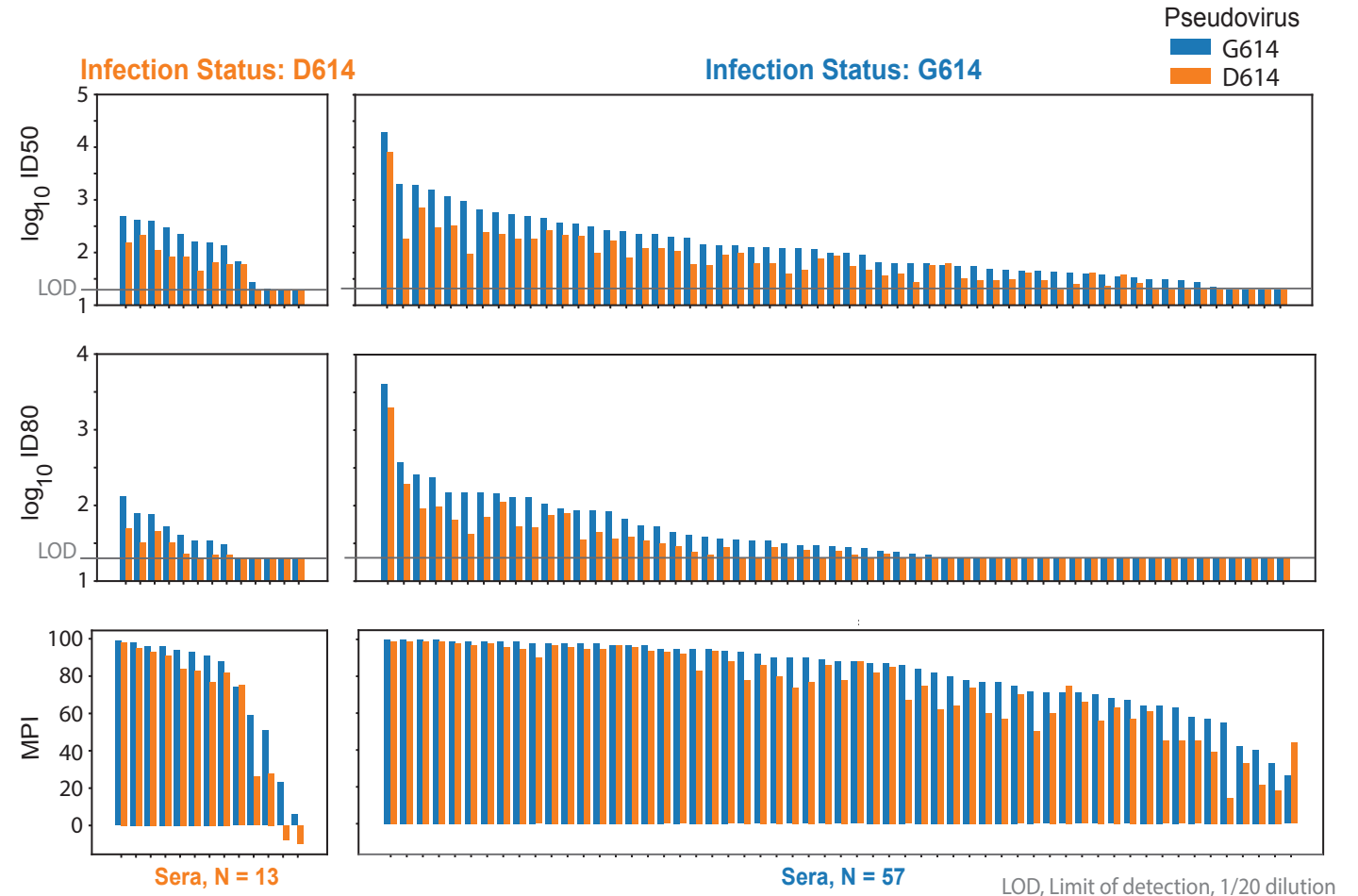
David Montefiori, Thushan de Silva (Sheffield)

Sera from 70 infections:

13 D614

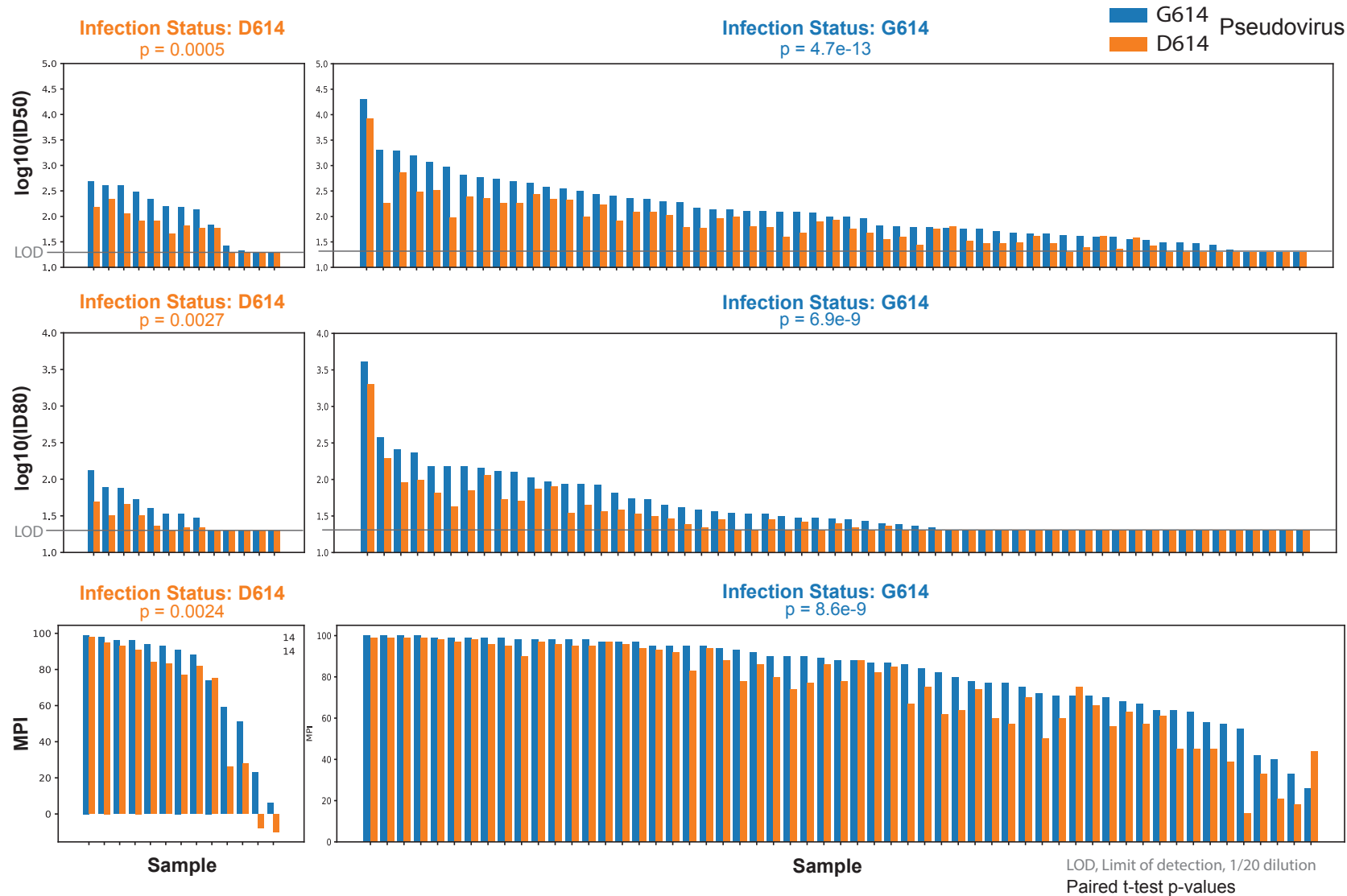
57 G614

There was no significant difference between the potency of responses in D614 and G614 infected individuals, but we only had 13 D samples.



Convalescent Sera infection status	ID50 Geometric mean		ID80 Geometric mean		Max. % Inhibition (MPI) mean	
	G:D ratio, (95% CI)	p-value	G:D ratio, (95% CI)	p-value	Δ G-D, (95% CI)	p-value
D614	2.0 (1.4 — 2.7)	0.00049	1.4 (1.2 — 1.7)	0.00272	11.8 (5.1 — 15.6)	0.0023
G614	1.9 (1.6 — 2.1)	4.7e-13	1.4 (1.3 — 1.5)	6.9e-09	7.9 (5.6 — 10.2)	8.56e-09

Same Data: note incomplete neutralization: MPI, maximum percent inhibition



RBD antibodies can be more potent against G614 than D614

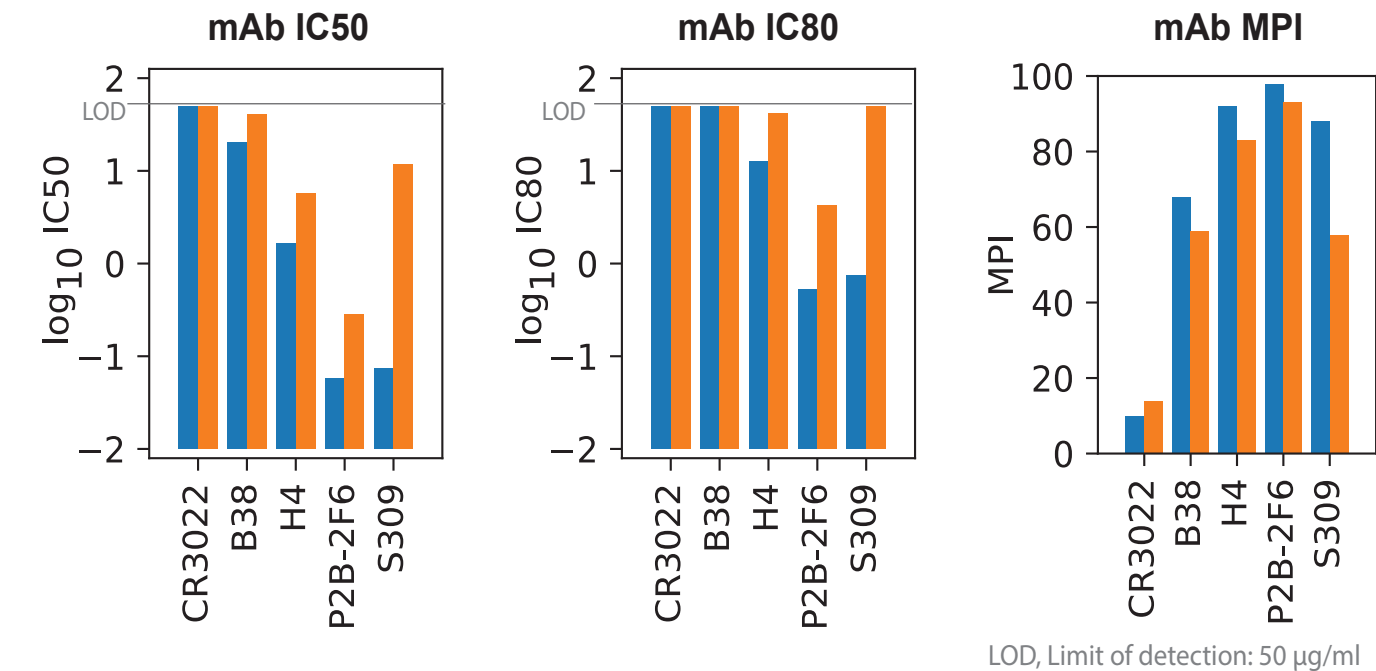
IC50 and IC80 values are in µg/ml, where a lower bar height corresponds to greater neutralization potency

MPI was calculated as the % neutralization at the highest mAb concentration tested.

CR3022, RBD binding, but non-neutralizing

S309 was isolated from a recovered SARS-CoV-infected subject, potentially cross-neutralizes SARS-CoV and SARS-CoV-2, binds outside the receptor binding motif of RBD and, based on cryo-EM structure, is not predicted to interfere with ACE2 binding (Pinto et al... Corti Nature 583, 290-295 (2020))

David Montefiori



RBD mAb	IC50 (µg/ml)			IC80 (µg/ml)			Max. % Inhibition (MPI)		
	D614	G614	D:G ratio	D614	G614	D:G ratio	D614	G614	Δ G - D
CR3022	>50	>50	na	>50	>50	na	14	10	na
B38	40.13	20.25	2.0	>50	>50	na	59	68	9%
H4	5.69	1.66	3.4	41.52	12.61	3.3	83	92	9%
P2B-2F6	0.28	0.057	4.9	4.26	0.52	8.2	93	98	5%
S309	11.83	0.073	162.0	>50	0.74	na	58	88	30%

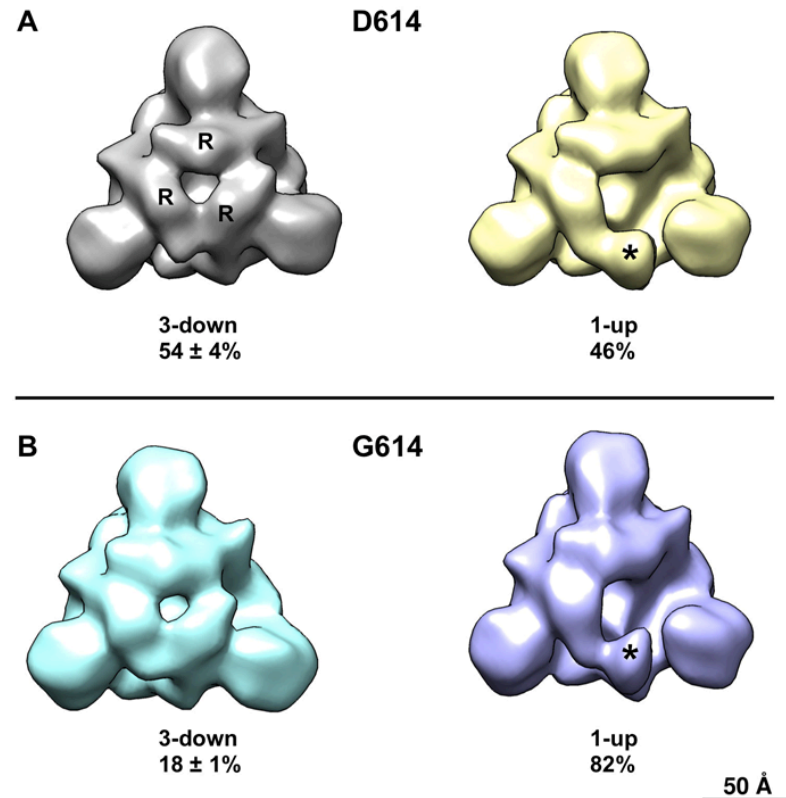
CR3022, B38, H4, P2B-2F6, and S309 were obtained from Dr. Peter Kwong

Mechanism: The **D614G** mutant Spike prefers the “one up” conformation which allows ACE2 interactions and exposes the RBD epitope regions

Negative stain electron microscopy reconstructions

Sophie Gobeil, Priyamvada Acharya

From Weissman et al.

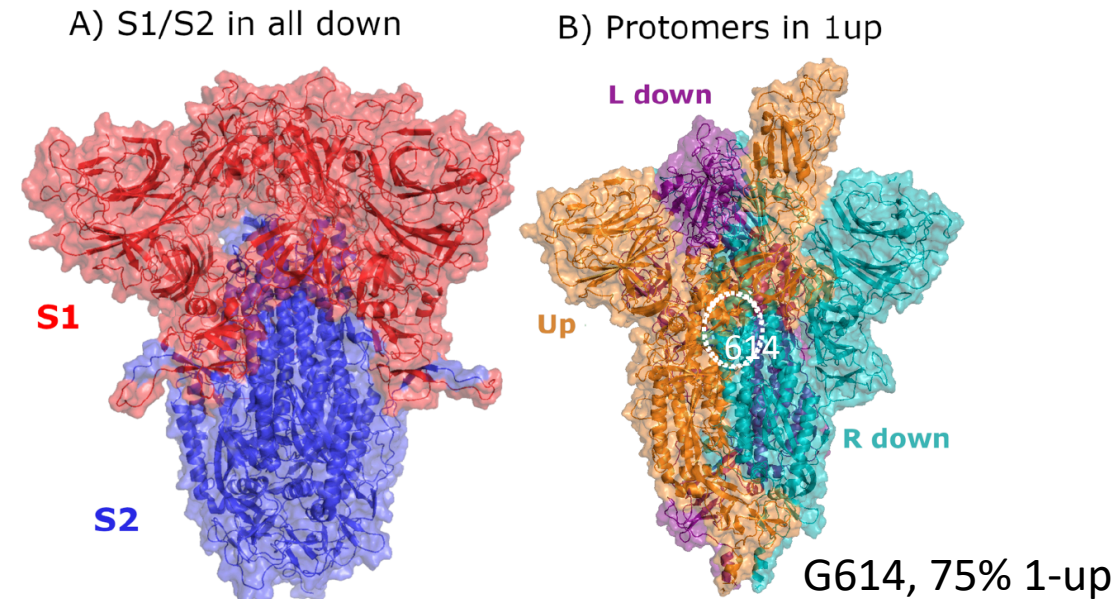


The SARS-CoV-2 Spike Variant D614G Favors an Open Conformational State

Rachael Mansbach, Srirupa Chakraborty, Kien Nguyen,

David Montefiori, Bette Korber, **S Gnanakaran**

bioRxiv



<https://biorxiv.org/cgi/content/short/2020.07.26.219741v1>

Timeline: defining the increased infectivity of G614 Spikes

Talk: April 3: "This is not a drill"

- 1) *G614 repeatedly increased in frequency in local regions*, and almost never vice versa
- 2) G614 took over in *even very well established D614 epidemics*



Clinical collaboration initiated



Experimental work initiated:

Pseudoviruses

Structures

Molecular Dynamics

Natural viruses

Immunological impacts

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BioRxiv Submission: April 30

- 1) More cases of regional G614 increased. We made cov.lanl.gov public, and found *several dozen of cases* where G614 increased, and only one exception, Iceland
- 2) G614 took over in even very well established D614 epidemics
- 3) G614 *is associated with higher viral load in upper respiratory tract*, but not with hospitalization, ~500 patients (*Thushan de Silva*)



Others started testing

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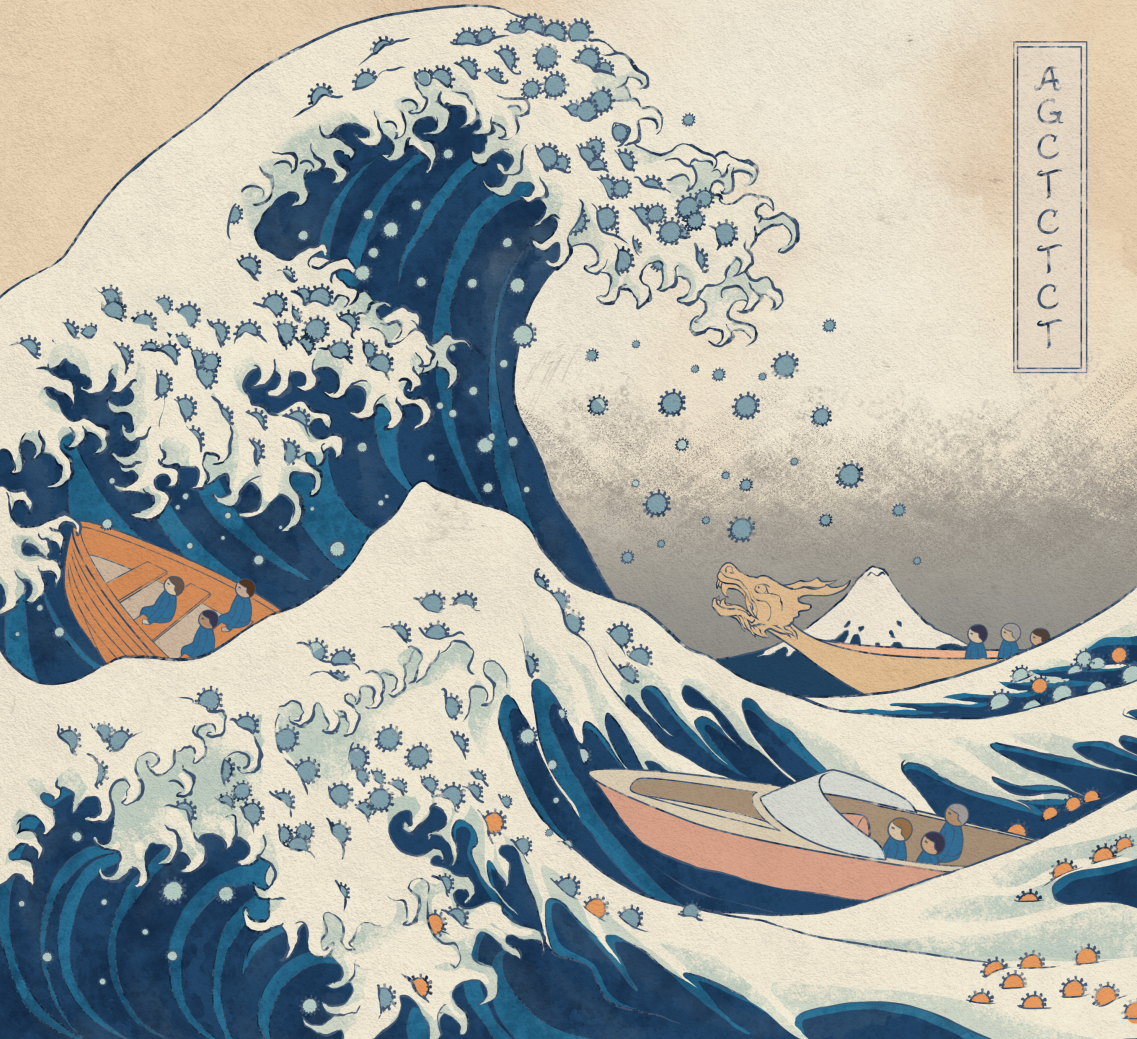
Others started testing

2.5 months

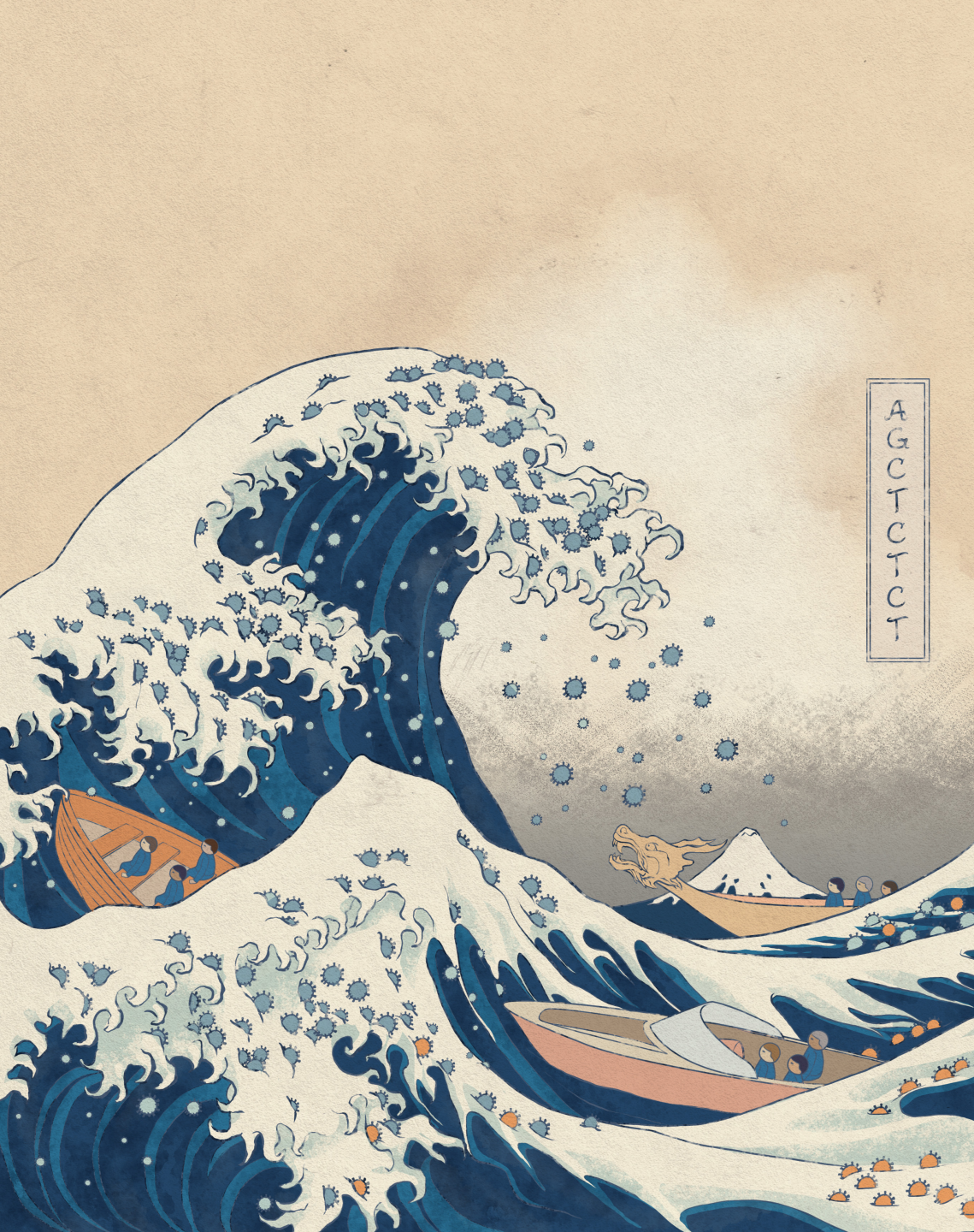
Cell preprint: final revisions, June 25

- 1) G614 repeatedly increased:
 - Developed systematic ways to explore **all regional data** in GISAID
 - Fisher's test: 48/50 cases
 - Isotonic regions: 60/64 cases
 - Explained several exceptions
- 2) G614 took over in even very well established D614 epidemics, and the frequency often continued increasing long after local stay home orders were in place
- 3) G614 is associated with higher viral load, ~1000 patients.
 - Confirmed in 2 preprints by others
- 4) G614 pseudoviruses are more infectious (Erica Ollmann Saphire & David Montefiori)
 - Confirmed 2 additional preprints
- 5) G614 sera neutralization sensitivity was not compromised
 - Confirmed in 1 additional preprint

In conclusion: Evidence so far that G614 is more transmissible



1. **The extreme repetition of the pattern of the shift from the D614 to G614.**
 1. If you toss a coin 83 times, and it comes up heads (G614) 80/83 times, perhaps its time to stop declaring it could be random chance
2. **This is not compatible with random founder effects because:**
 1. Even repeat introductions of G614 would not be able to *repeatedly* overwhelm well-established D614 epidemics
 2. Super-spreader events or random biases should not always favor the G614 form, if both forms are equally likely to propagate
 3. The gain in G614 often continues for weeks after stay at home orders are implemented.
3. **Increased infectivity of G614 Spikes in a pseudotype assay**
 - Now repeated in at least 4 different laboratories
4. **Lower PCR Ct in G614 infections indicative of higher viral**
 - Now repeated in at least 3 different laboratories
5. **Mechanism for increased infectivity is beginning to be defined: G614 favors a one up conformation that makes the RBD more accessible for ACE2 receptor binding.**
 1. Gnana Gnanakara et al.
 2. Drew Weissman et al.
 3. Yurkovetskiy et al., BioRxiv



Why does this matter?

1. We need to understand the biology of this virus

2. The virus is different now, and more transmissible than it was when the world began to shelter in place mid-March

- G614 is in itself an inspiration for greater caution
- It could help inform epidemiological models

3. We need to know the impact (good or bad) of G614 on vaccines and therapeutics

- G614 S is sensitive to polyclonal antibodies from sera (Saphire)
- G614 S is actually more sensitive to D614 vaccine raised Nabs than is D614 (Weissman, Montefiori)
- G614 S's greater infectivity makes it easier to use in a pseudovirus assay than D614 (Montefiori)
- G614 may be useful as a *vaccine antigen* because the RBD, a key neutralizing target, is more exposed.

Acknowledgments: preprint, Cell:

Tracking SARS-CoV-2 Spike mutations: evidence for increased infectivity of D614G

Key players:

Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, **Hengartner N**, Giorgi EE, Bhattacharya T, Foley B, **Hastie KM, Parker MD**, Partridge DG, Evans CM, Freeman TM, **de Silva TI**, McDanal C, Perez LG, Tang H, Moon-Walker A, Whelan SP, **LaBranche CC, Sapphire EO**, and **Montefiori DC**

The Sheffield COVID-19 Genomics Group:

Angyal A, Brown RL, Carrilero L, Green LR, Groves DC, Johnson KJ, Keeley AJ, Lindsey BB, Parsons PJ, Raza M, Rowland-Jones S, Smith N, Tucker RM, Wang D, Wyles MD

Support:

COG-UK CONSORTIUM, MRC UK Research & Innovation (UKRI), NIHR and Genome Research Limited, the Wellcome Sanger Institute, NIHR Sheffield Biomedical Research Centre (BRC), Los Alamos National Laboratory Directed Research NIH NIAID, DHHS, CoVIC of the COVID-19 Therapeutics Accelerator, Bill and Melinda Gates Foundation, Mastercard, private philanthropic support, the Overton family, and Emergent Ventures in aid of COVID-19 research.

Particularly thoughtful colleagues:

Andrew McMichael, Xiao-Ning Xu, Anthony West, Barbara Imperiali, Rachael Mansbach, Srirupa Chakraborty and Kien Nguyen

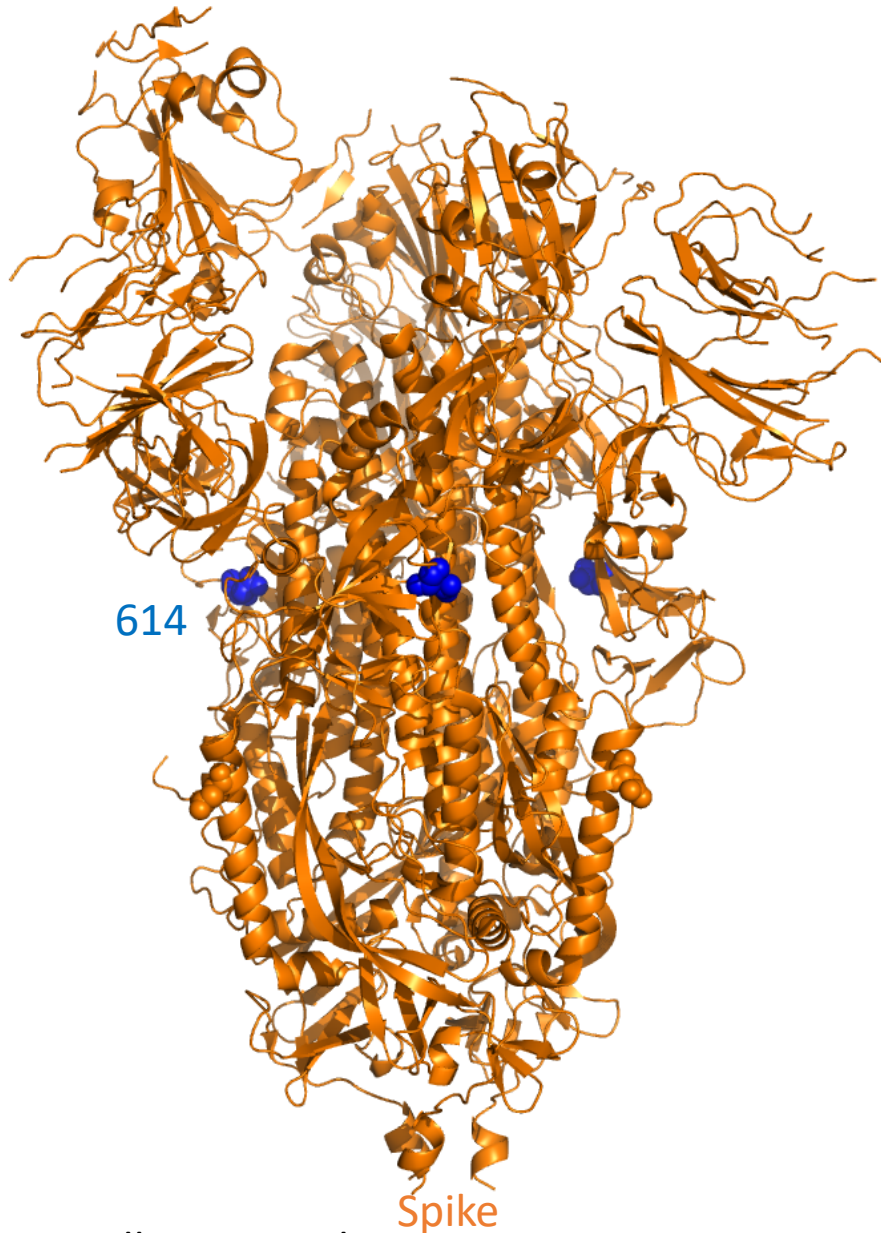


We gratefully acknowledge the team at GISAID for creating SARS-CoV-2 global database, and the many people who provided sequence data

Artwork by SciStories LLC (blue wave) and Christina Corbaci (map of the world)

Extras

Can a single amino acid change a phenotype?



There are *many* examples in the scientific literature.

I. Immune escape, SARS-CoV-1 example:

Broadening of Neutralization Activity to Directly Block a Dominant Antibody-Driven SARS-Coronavirus Evolution Pathway

Sui et al. PLoS Pathog. (2008) 4: e1000197

- RDB mutation arises between the 2002/3 and 2003/4 in civet cats and human
- Confers resistance to the potent RDB targeting NAb 80R
- The escape mutation is recapitulated in vitro

II. Enhanced infectivity, HIV-1 example:

A signature in HIV-1 envelope leader peptide associated with transition from acute to chronic infection impacts envelope processing and infectivity.

Asmal et al. PloS One (2011) 6:e23673

III. Host specificity, HIV-1 example:

Envelope residue 375 substitutions in simian-human immunodeficiency viruses enhance CD4 binding and replication in rhesus macaques

Li et al. PNAS USA (2016) 113:E3413

Evaluating the effects of SARS-CoV-2 Spike mutation D614G on transmissibility and pathogenicity

Volz et al. medRxiv 2020.07.31.20166082; doi: <https://doi.org/10.1101/2020.07.31.20166082>

Table 1 | Estimates of the selection coefficient favouring the Spike 614G variant using different sources and methods.

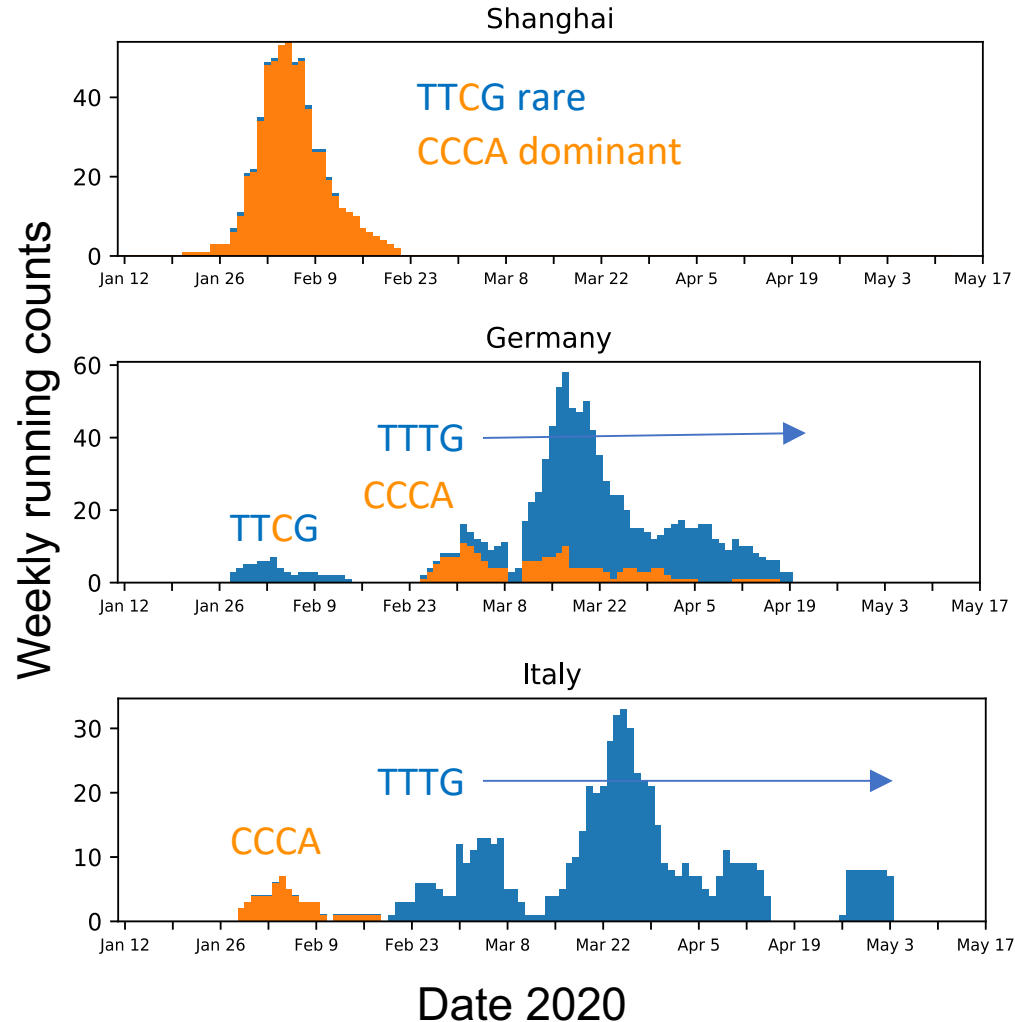
Method	Selection coefficient
Logistic	0.21 (0.06, 0.56) ¹
Exp/Exp coalescent	0.29 (-0.24, 1.18) ²
Skygrowth coalescent	0.17 (-0.24, 0.57) ²
London SEIR structured coalescent	0.10 (-0.15, 0.41) ²
London SEIR with importance sampling	0.26 (-0.01, 0.58) ²

¹maximum likelihood estimate (95% confidence interval)

²median posterior (95% credible interval)

Origins: G614 is almost always part of a clade defined by 4 bases

Early cases of the G clade



G-clade mutations (C3037T, C14408T, A23403G) CCA -> TTG
Plus the linked mutation in the UTR: C241T CCCA -> TTTG

11805	TTG	(72.03%)	9692	TTTG	(71.65%)
4582	CCA	(27.96%)	3835	CCCA	(28.35%)

Variants:

53	CTG	} 0.76%	51	TCTG	5	CTCG
39	TCG		32	TTCG	4	CCTA
16	CCG		13	CTTG	3	TCTA
9	TTA		11	TCCA	2	CTTA
8	CTA		9	TCCG	2	CTCA
5	TCA		7	CCCG	1	TTCA
1	ACA		6	TTTA	1	CCTG

Earliest examples in GISAID:

TTTG: Germany, Jan 2020: cluster of cases late Jan.-Feb.

One example: Germany/BavPat1/EPI_ISL_406862|2020-01-28

TTTG: Sampled several times in China, e.g.:

Sichuan/SC-PHCC1-022/EPI_ISL_451345|2020-01-24

Shanghai/SH0025/EPI_ISL_416334|2020-02-06

Guangzhou/GZMU0019/EPI_ISL_429080|2020-02-05

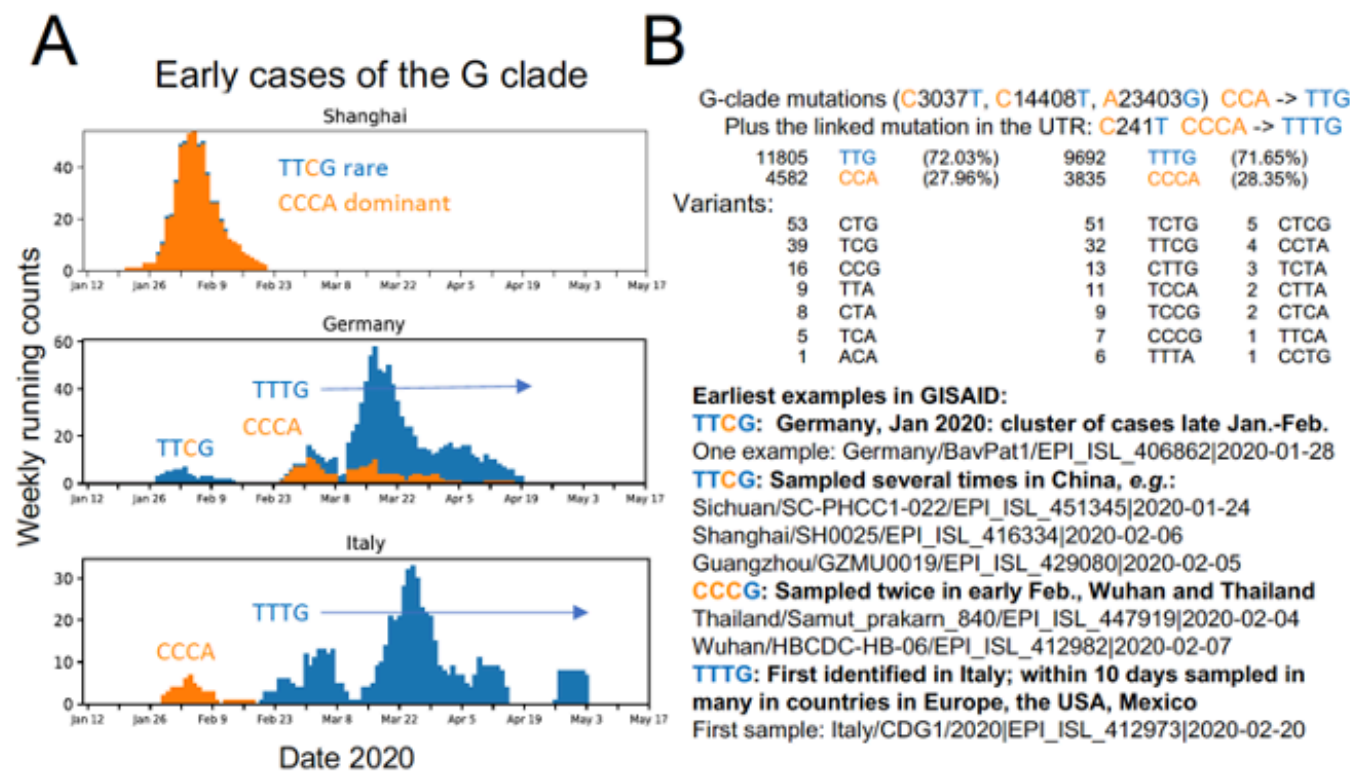
CCCG: Sampled twice in early Feb., Wuhan and Thailand

Thailand/Samut_prakarn_840/EPI_ISL_447919|2020-02-04

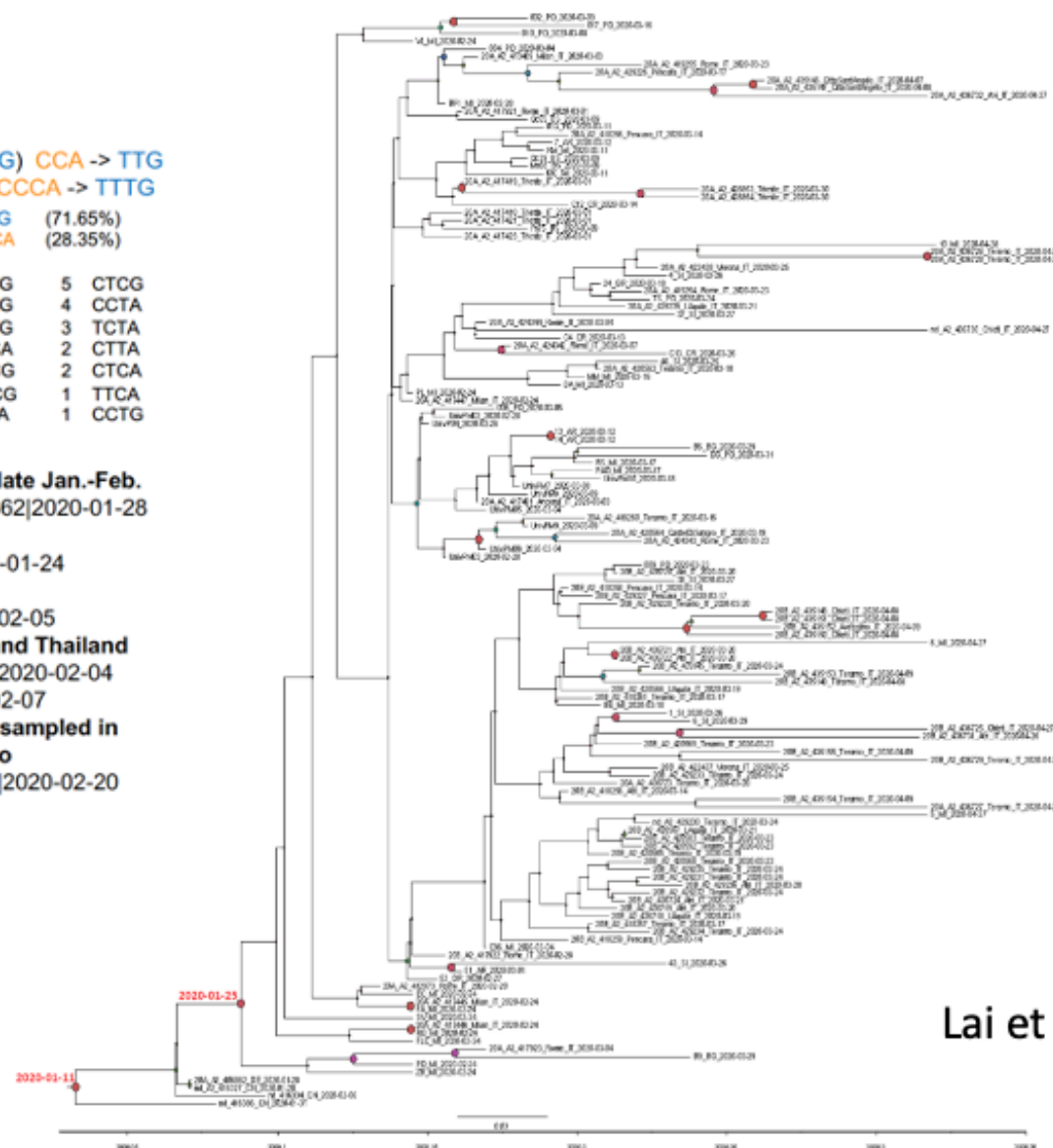
Wuhan/HBCDC-HB-06/EPI_ISL_412982|2020-02-07

TTTG: First identified in Italy; within 10 days sampled in many in countries in Europe, the USA, Mexico

First sample: Italy/CDG1/2020|EPI_ISL_412973|2020-02-20



Korber et al., Cell 2020, supplementary figure.

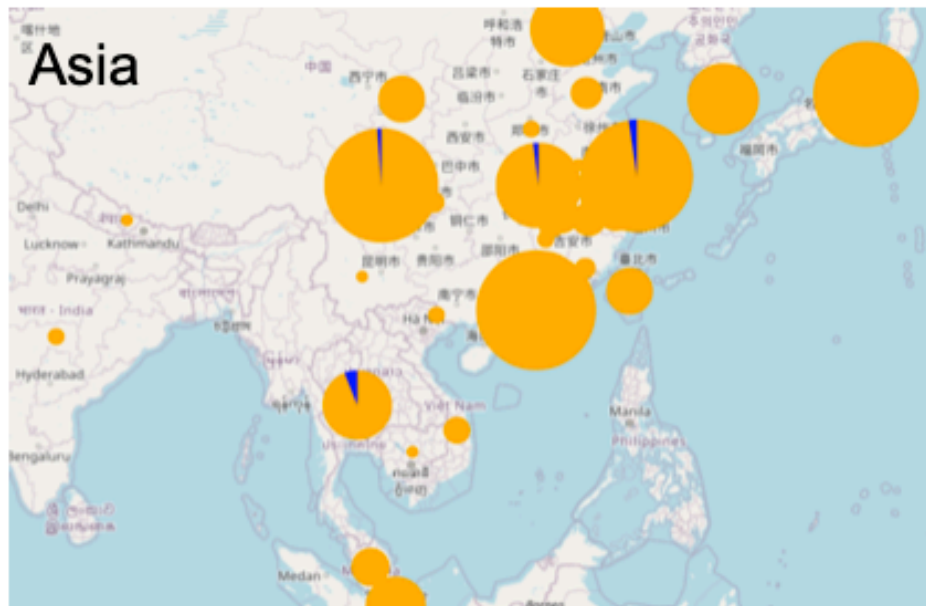


Lai et al.

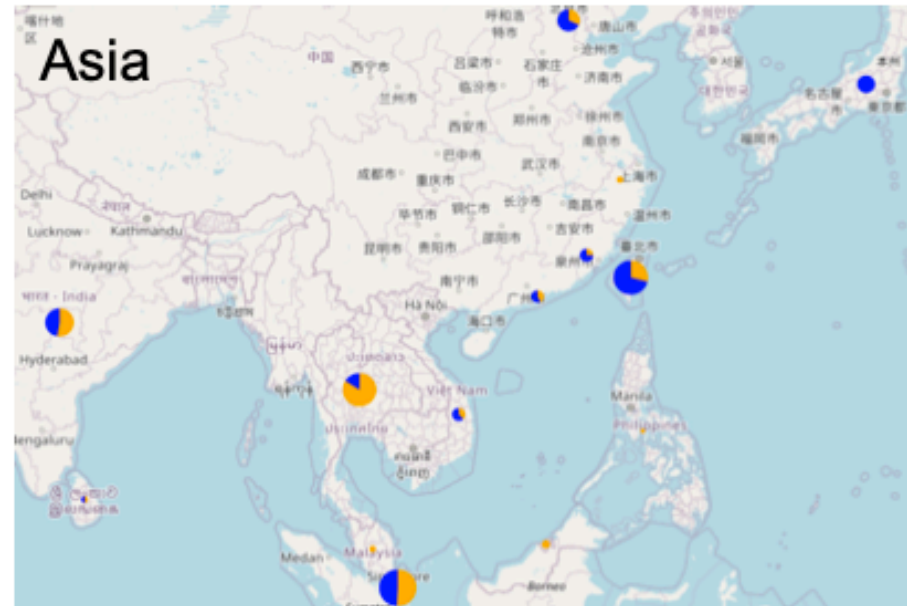
Figure 2. SARS-CoV-2 tree of 136 Italian strains plus one German and three Chinese isolates from Shanghai, showing statistically significant support for clades along the branches (posterior probability > 0.7). Large red and purple circles indicated highest posterior probability. Calendar dates of the tree root and the Italian clade were showed in red.

Lai A, Bergna A, Caucchi S, Clementi N, Vicenti I, Dragoni F, et al. Molecular tracing of SARS-CoV-2 in Italy in the first three months of the epidemic. doi:10.1101/2020.07.06.20147140

Prior to March 1



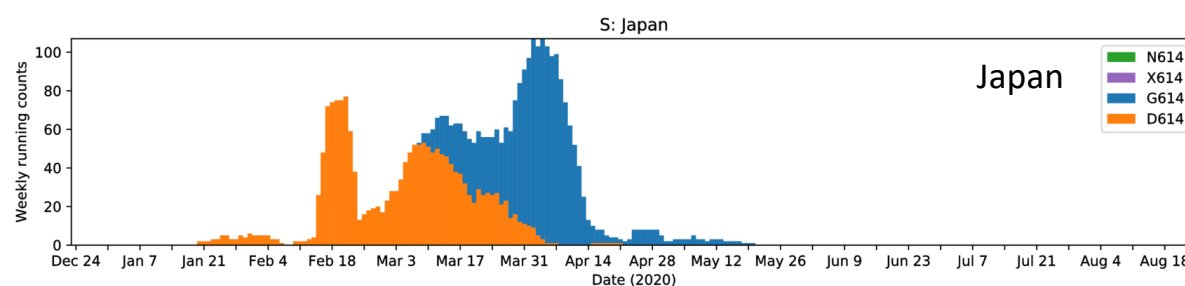
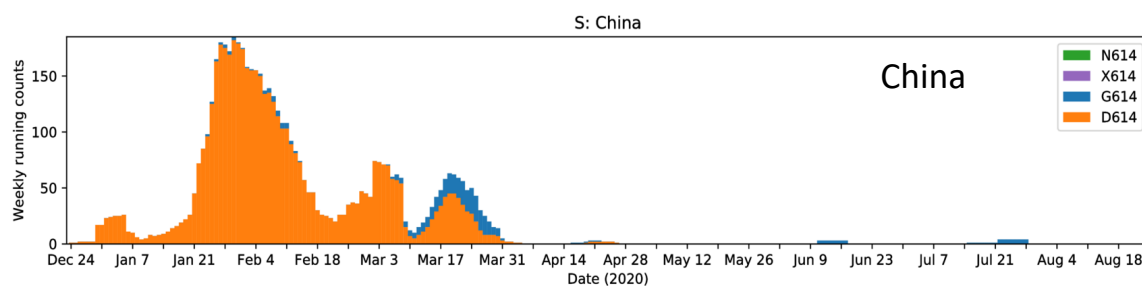
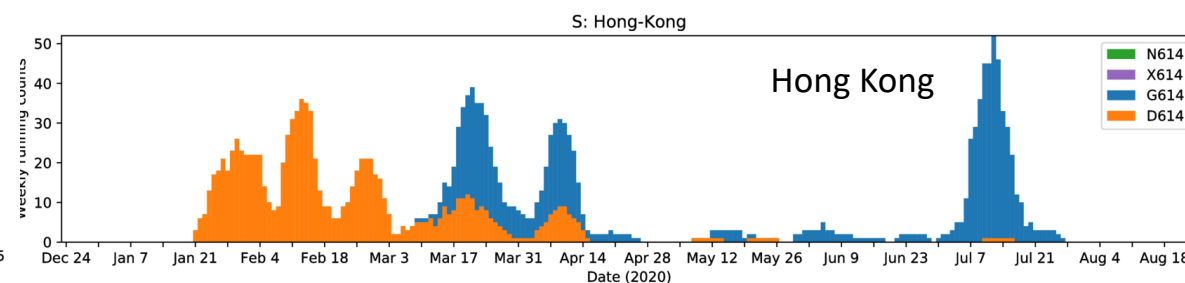
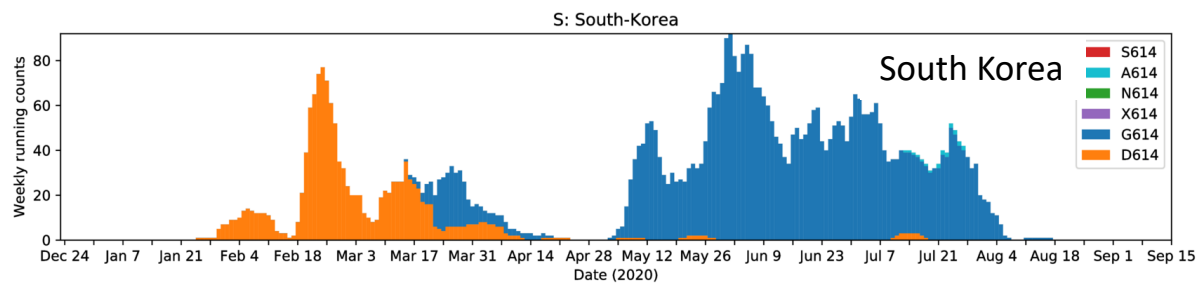
March 11-20



Poor recent sampling

Shift is delayed in parts of Asia, but is beginning

08/21/2000 update



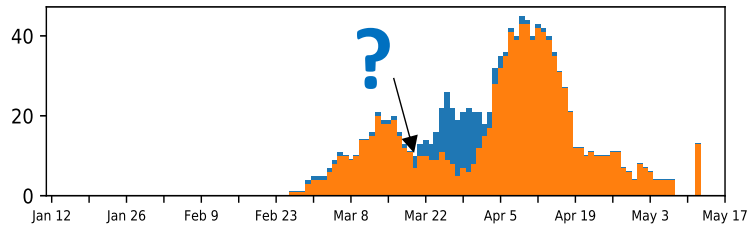
One of two exceptions: Santa Clara county

Sampling May 29, 2020

Santa Clara County

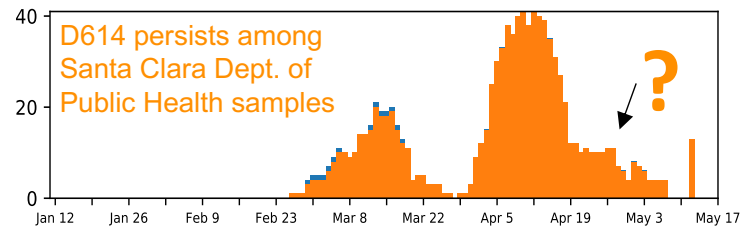
Nearly all G614 samples are labeled "Stanford",
suggesting a local cluster with Santa Clara county.

Santa Clara



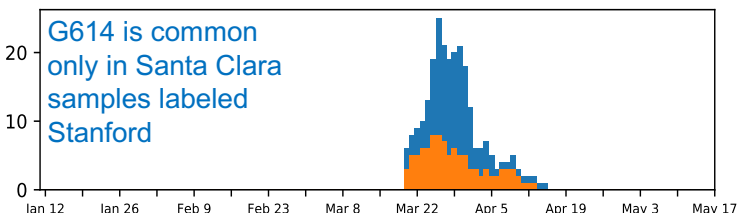
Santa Clara Public Health

D614 persists among
Santa Clara Dept. of
Public Health samples



Santa Clara Stanford

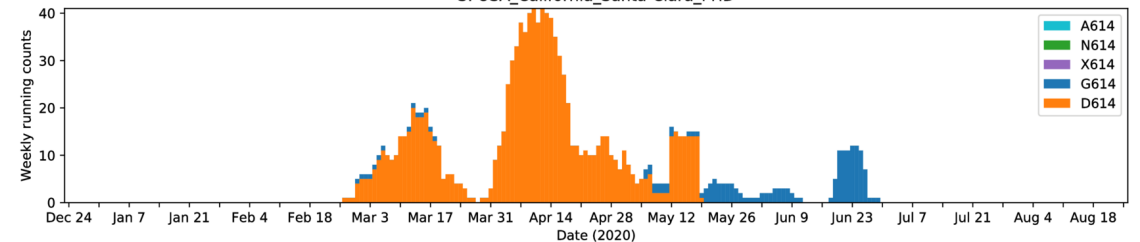
G614 is common
only in Santa Clara
samples labeled
Stanford



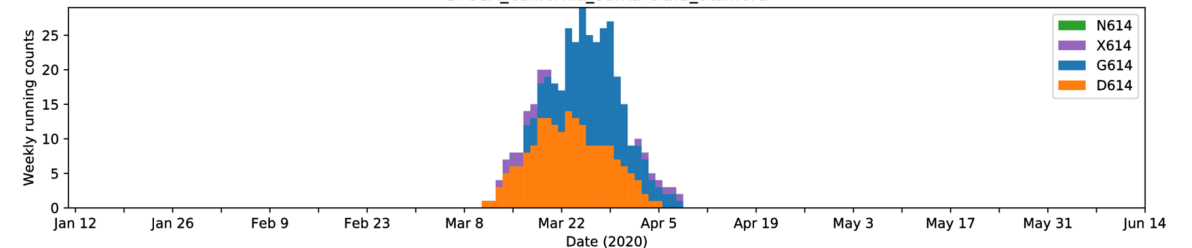
Sampling update July 20, 2020

There is evidence for the shift to G614 from both locations

S: USA_California_Santa-Clara_PHD



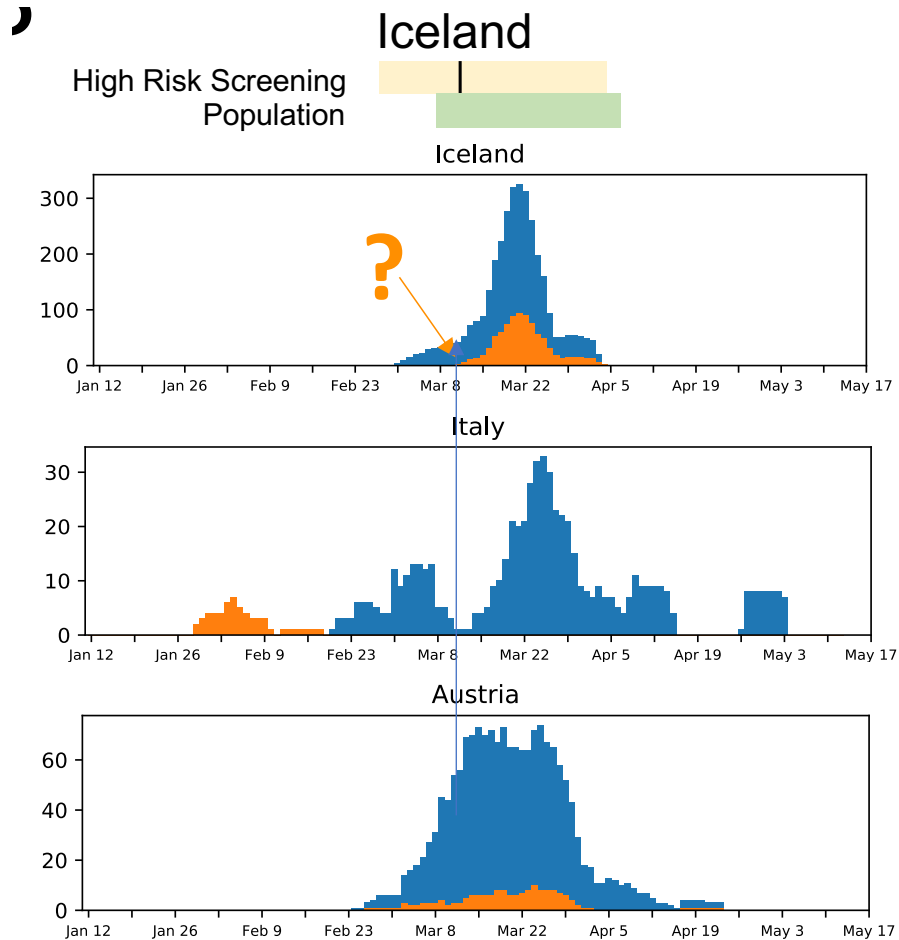
S: USA_California_Santa-Clara_Stanford



Second of two exceptions: Iceland

Gudbjartsson et al., Spread of SARS-CoV-2 in the Icelandic Population
NEJM 382:2302 2020

Sampling May 29, 2020



G614 dominated early samples:

- Through mid-March: Targeted testing, the majority of were travelers to were high risk places, most from G614 prevalent Italy and Austria

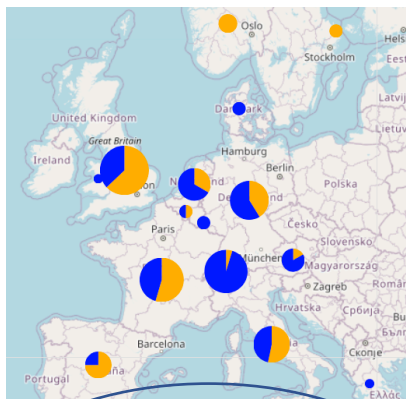
D614 begins to be sampled ~March 13

- Local population screening began March 13,
- Travelers from the US and UK were included in targeted testing with D614/G614 mixed populations began March 15.

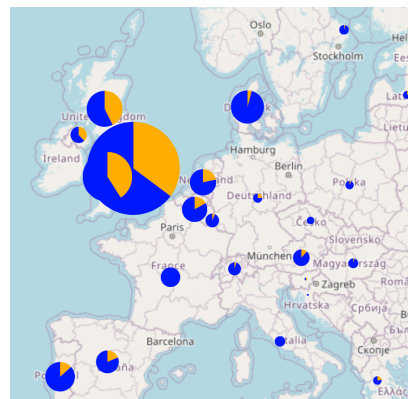
No recent samples from Iceland

A

Prior to March 1



March 21-30

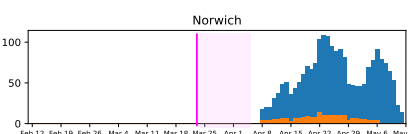
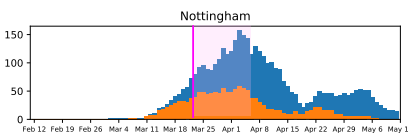
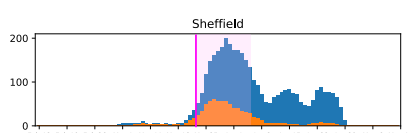
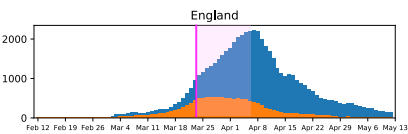
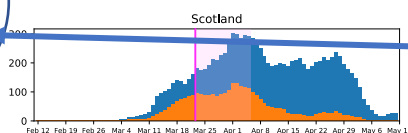
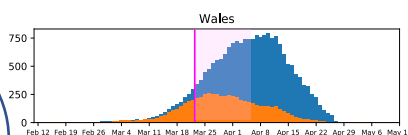
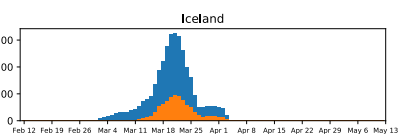
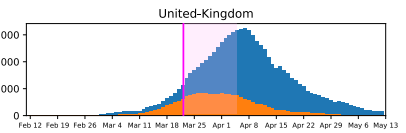
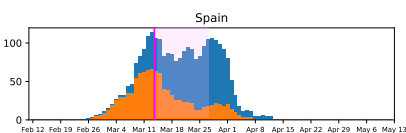
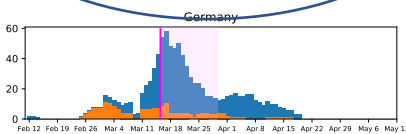
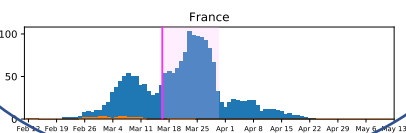
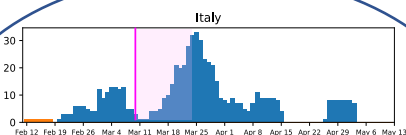


■ S D614
■ S G614

Europe

B

Weekly running counts



Date 2020

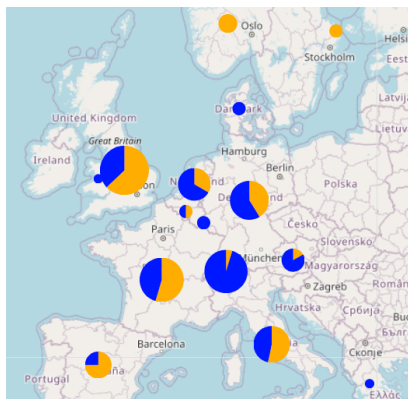
Date 2020

Some countries began early expansion with G614:

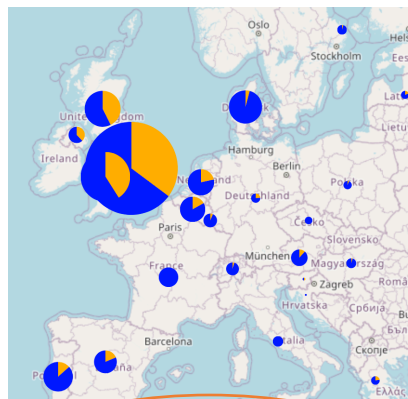
Italy, France

A

Prior to March 1



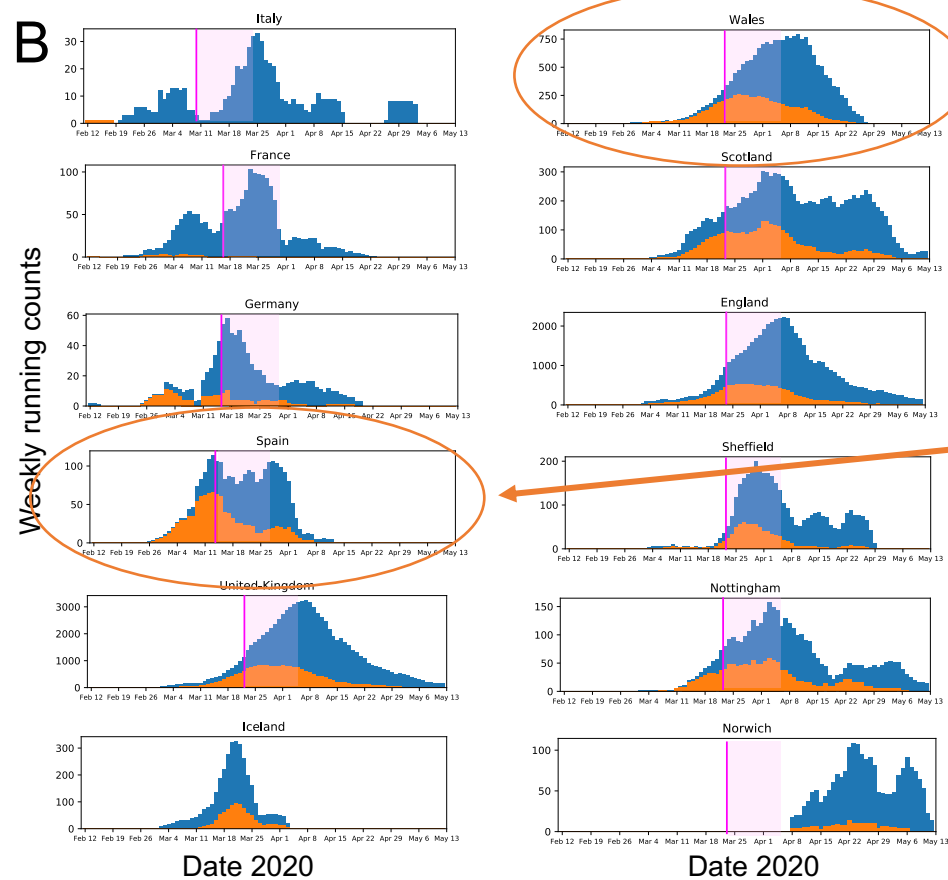
March 21-30



■ S D614
■ S G614

Europe

B

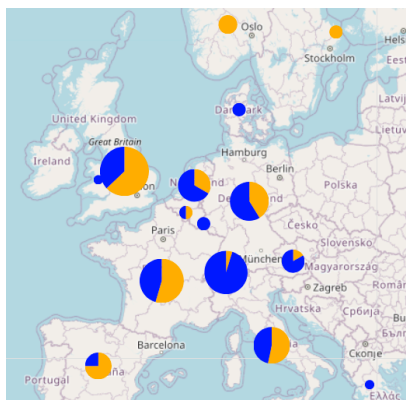


Some countries began early expansion with G614:
Italy, France

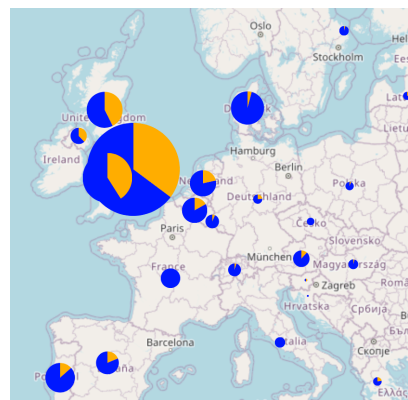
Others had well established D614 prior to the expansion of G614
Spain, Wales

A

Prior to March 1



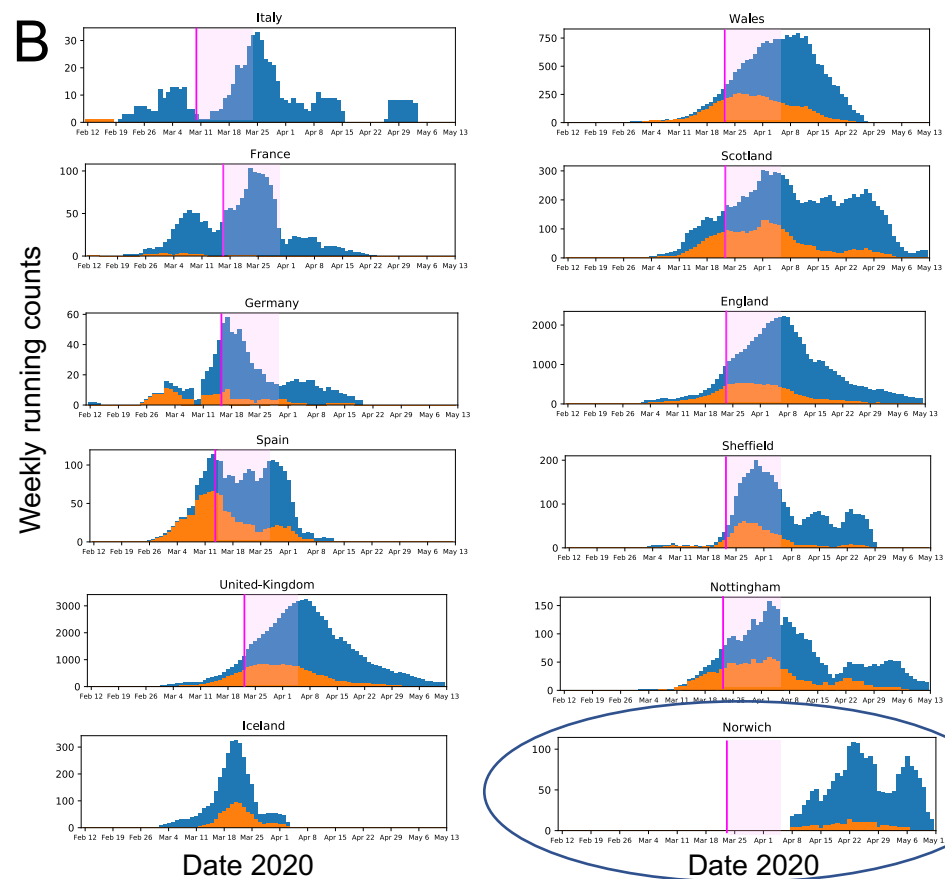
March 21-30



■ S D614
■ S G614

Europe

B



Some countries began early expansion with G614:

Italy, France

Others had well established D614 prior to the expansion of G614

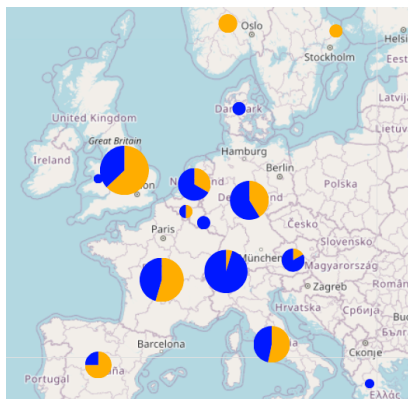
Spain, Wales

Some places sampling started late Spring, almost all G614

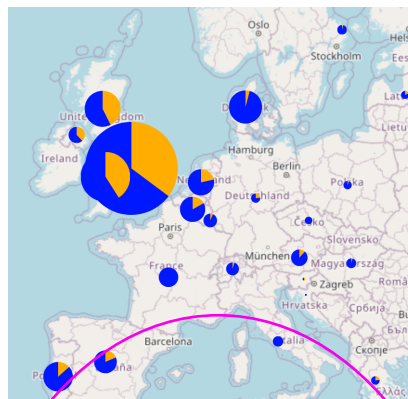
Norwich

A

Prior to March 1



March 21-30



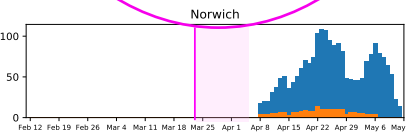
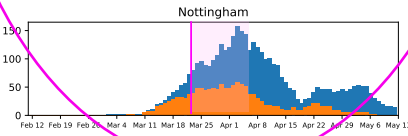
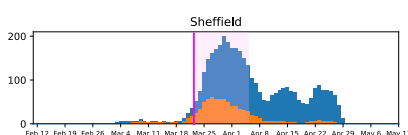
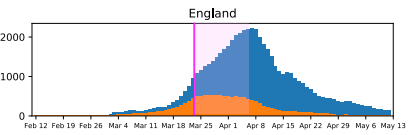
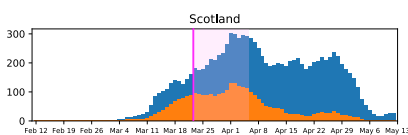
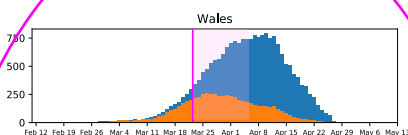
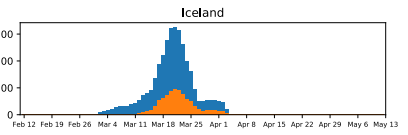
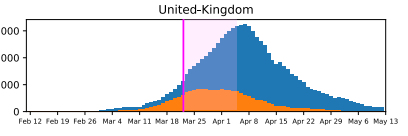
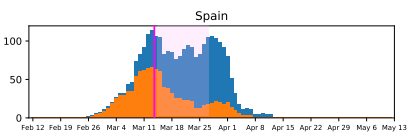
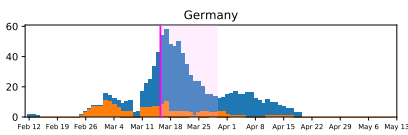
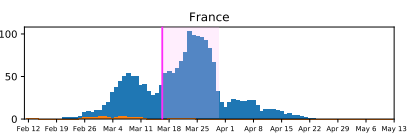
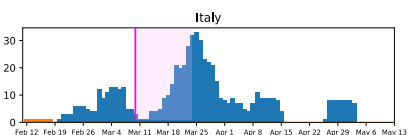
■ S D614
■ S G614

Europe

In many places the increasing prevalence of the **G614** form continued well after stay-at-home orders.

B

Weekly running counts



Date 2020

Date 2020

OCEANIA: VERY WELL ESTABLISHED D614 SHIFTS TO G614

